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## IDENTITY OF THE TOXINS OF DIFFERENT STRAINS OF BACILLUS WELCHII AND FACTORS INFLUENCING THEIR PRODUCTION IN VITRO.

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(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, September 1, 1917.)

It has been reported in an earlier publication<sup>1</sup> that under suitable cultural conditions *Bacillus welchii* produces an exotoxin which is comparable in its physical and biological properties with diphtheria and tetanus toxins. The original study was limited to five strains of the organism, all of which yielded toxins which were qualitatively identical although differing quantitatively. The identity of the various toxins was determined by the observation that an antitoxin produced with a toxin from any strain neutralized the toxins from all the other strains. Moreover, it was found that such a monovalent antitoxin would protect against and control infections with any of the strains included in the study. The small number of strains studied and the closely related sources of four of the five made it desirable to extend the experiments before concluding that all strains of *Bacillus welchii* produce the same toxin. From the standpoint of developing a specific therapy, it was essential to know whether one toxin is common to all strains, regardless of their origin. To ascertain this, twenty-two other strains have been collected from widely different sources and studied with reference to the identity of their toxins.

### *Source of the Strains.*

Two of the new strains were part of the cultures isolated during the summer of 1916 on the Western Battle Front by Dr. J. P. Simonds of Chicago. Four strains were obtained from the Pasteur Institute through the kindness of Dr. Carrel. Dr. Carrel also brought us necrotic tissue from two cases of severe gaseous gangrene, from which

<sup>1</sup> Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.



we isolated the infecting strains. Two strains were isolated from cases of gaseous gangrene occurring in New York City. Five strains were obtained through the kindness of Dr. F. M. Allen from spontaneous gas bacillus infections in diabetic dogs, two others from dog feces, two from human feces, two from market milk, and one from garden soil.

All the strains were obtained in pure culture as indicated by morphological, cultural, and pathogenetic properties, and each strain was tested separately, without animal passage, for artificial toxin production.

#### *Production and Identification of the Toxins.*

Each strain was grown in 0.2 per cent glucose muscle broth for 20 hours at 37°C., and the cultures were centrifuged and filtered.<sup>1</sup> The filtrates were tested for toxicity by intramuscular injection in pigeons. The same quantity of filtrate that was used in the toxicity tests was mixed respectively with *Bacillus welchii* antitoxin and an equal amount of normal serum of the same animal. The mixtures were incubated for 30 minutes at 37°C. and then injected into the breast muscles of pigeons. The degree of toxicity of the filtrate and of the serum-filtrate mixtures was calculated from the degree of the local edema and necrosis which ensued and the quantity of filtrate necessary to cause death.

The procedure described established the fact that all the strains produced toxins which were indistinguishable in pathologic effects from those produced by the five strains described in our first publication, and that an antitoxin made with toxin from one of the former strains completely neutralized the toxins from all the new strains. The toxins of the individual members of the series exhibited, however, a wide range of potency; the lethal dose for pigeons on intramuscular inoculation varied from 0.3 cc. to 3 cc. The lesions and toxic effects were, nevertheless, of the same quality. The toxin-producing power of the less active strains was materially increased by animal passage, and an indication was obtained that a direct relation between infectiosity and toxin production exists. However, the latter point demands a more systematic study than we have yet been able to give it.

To summarize, we may state that twenty-two new strains of *Bacillus welchii* have been secured from widely different sources and studied with regard to morphological, cultural, and pathogenetic properties and toxin production. They have by these means been identified as typical members of the *Bacillus welchii* group of bacilli. Moreover, each strain produced a toxin which was qualitatively indistinguishable in physical and biological properties from the toxins produced by any other members of the series, although the different toxins manifested varying degrees of potency.

Up to the present time we have studied twenty-seven strains of *Bacillus welchii* from widely different sources and have found all to produce a toxin in common.

### *Factors Influencing the Artificial Production of Bacillus welchii Toxin.*

In our first publication<sup>1</sup> the following method was given for obtaining the toxin *in vitro*: To plain beef infusion broth in 10 cc. quantities in test-tubes are added several fragments of sterile skeletal muscle of the pigeon or rabbit. The tubes, having been proved sterile, are inoculated with *B. welchii*, overlaid with paraffin oil, and enclosed in a vacuum jar from which the oxygen is then exhausted. After an incubation of from 18 to 24 hours, the contents of the culture tubes are centrifuged and the fluid portion of the culture is passed through a Berkefeld N candle. The filtrate contains the toxin. It was stated also that to obtain the most potent product not more than 0.1 per cent glucose should be present, and that the incubation should not exceed 24 hours.

Since then we have been able to carry out a more extensive and systematic investigation of the influence of various factors on artificial toxin production.

Certain technical precautions are necessary in determining the influence of different factors on the production of the toxin *in vitro* in order to eliminate extraneous influences. In the first place, the culture with which the media are inoculated must be controlled with regard to age, the medium in which it is grown, and the number of artificial generations since animal passage. Then the different lots of medium in which toxin is to be produced for the purpose of comparison should be inoculated simultaneously and with the same quantity of culture. Errors may arise in the process of collecting the filtrates. A portion of the toxin is always held back by the filter-

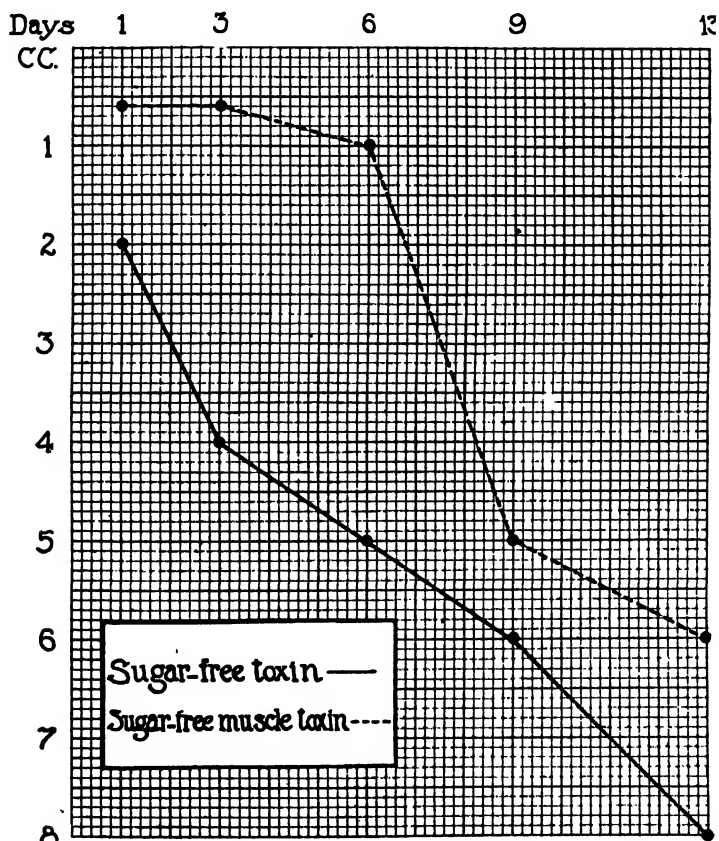
ing candles, and if they are not perfectly clean and open, a large percentage of the toxin may be retained. Under the best conditions different candles seem to retain different amounts of toxin. The following procedure has been followed in this work: A good toxin-producing strain (617 d, Simonds) was used. The strain was of maximum virulence as the result of a number of animal (pigeon) passages. A pigeon was inoculated with an acutely lethal dose of the culture early in the morning of the day preceding the beginning of an experiment. Immediately after the death of the animal, usually late in the afternoon, tubes of plain beef infusion broth were inoculated with small fragments of the infected muscle and incubated at 37° C. over night. The test media were then inoculated with definite quantities of the bouillon cultures, exhausted in a vacuum jar, and incubated in the usual way. At the end of the planned incubation period, the contents of the culture tubes were centrifuged until the supernatant fluid was free of solid matter. The fluid was then syphoned off and filtered. The filters were proved to be open by testing them with sterile distilled water immediately before use and were recleaned as soon as there was evidence of obstruction of the flow of the fluid through them. In this way consistent results were obtained.

In order to test the potency of the toxin, three different quantities of each toxic product were injected into the breast muscles of three pigeons respectively. The degree of toxicity was estimated from the extent of the edema and necrosis, when sublethal amounts were given, and from the minimal lethal dose. In a number of instances, where the degree of toxicity could not be approximately predicted, the largest quantities given did not cause death or even local lesions. This was especially true of the filtrates from the cultures containing the higher percentages of glucose, in which instances only relative values were obtained.

*Influence of Fresh Muscle and Incubation Time on the Potency of the Toxin.*

We had observed more or less incidentally that the most potent filtrates were obtained after the cultures had been incubated from 18 to 24 hours, and that an extension of the incubation period caused

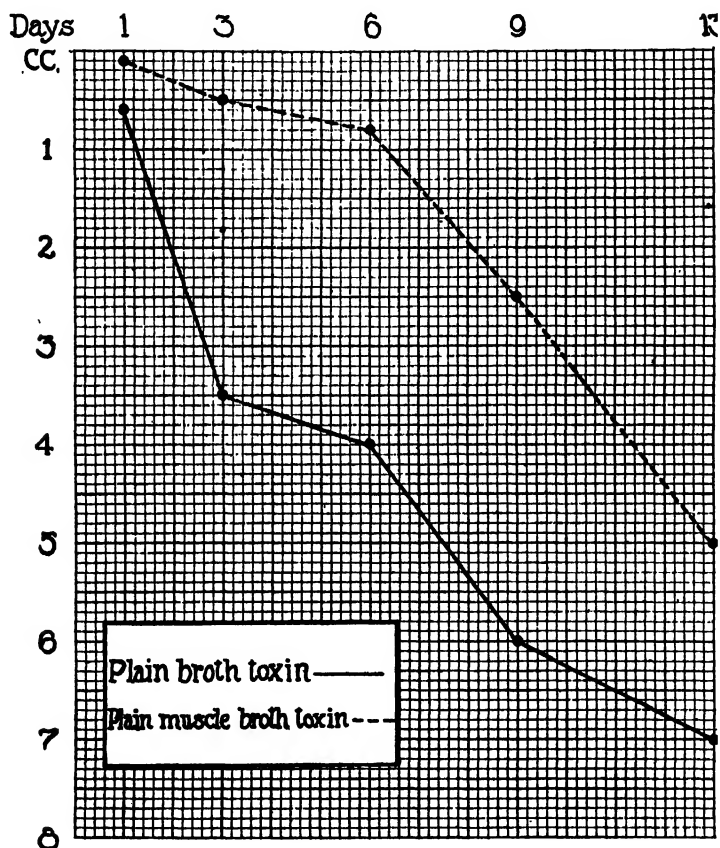
a decrease in toxicity; but the rate and the extent of the decrease in toxicity as the incubation time is prolonged had not been determined. It was, therefore, considered desirable to determine more accurately the relation between incubation time and toxicity. With this end



TEXT-FIG. 1. The media were sugar-free broth and sugar-free broth to which fresh sterile muscle had been added. The tubes were inoculated, freed of oxygen, and incubated in the usual way. The two products are designated as sugar-free toxin and sugar-free muscle toxin respectively.

All the text-figures have the same arrangement. The incubation time of the different specimens of filtrate is represented on the ordinates, while the smallest quantities necessary to produce local lesions and death are represented on the abscissæ. The height of the curves from the base-line represents, therefore, the degree of toxicity.

in view, a variety of media, consisting of various kinds of beef infusion, was inoculated with cultures of *Bacillus welchii* and the filtrates were collected and tested at different intervals, ranging from 16 hours to 13 days.

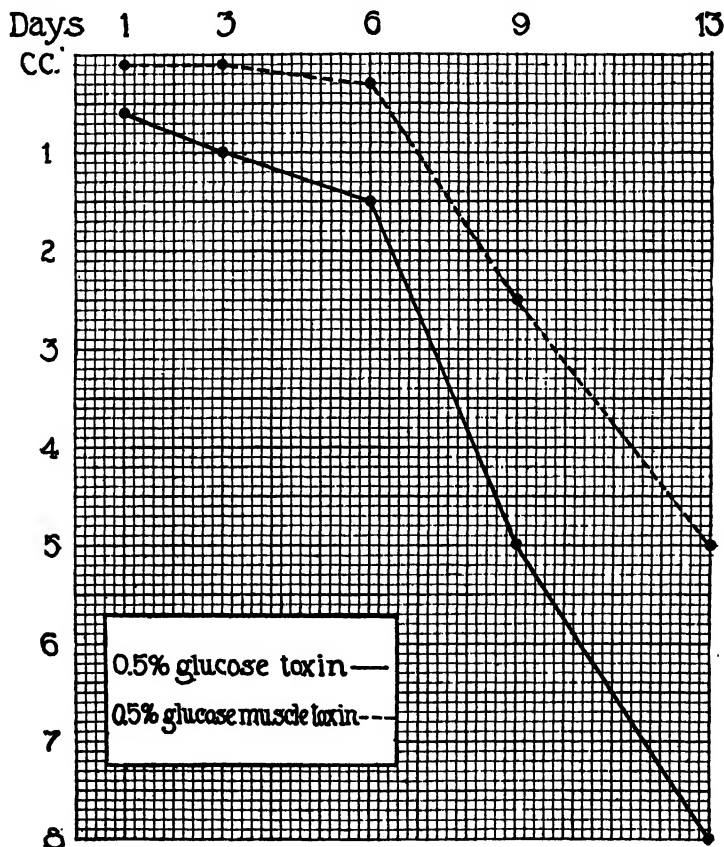


TEXT-FIG. 2. The media were plain broth and plain broth to which sterile muscle had been added. The filtrates are designated as plain broth toxin and as plain muscle broth toxin.

The results obtained may be summarized in the statement that the toxicity of the filtrates is inversely proportional to the incubation time, calculating from the end of the 1st day, and that this general relation obtains independently of the nature of the medium. The

rapidity of the decrease in toxicity, however, is materially influenced by the percentage of glucose in the medium and the presence of raw muscle. The results are presented graphically in Text-figs. 1 to 5.

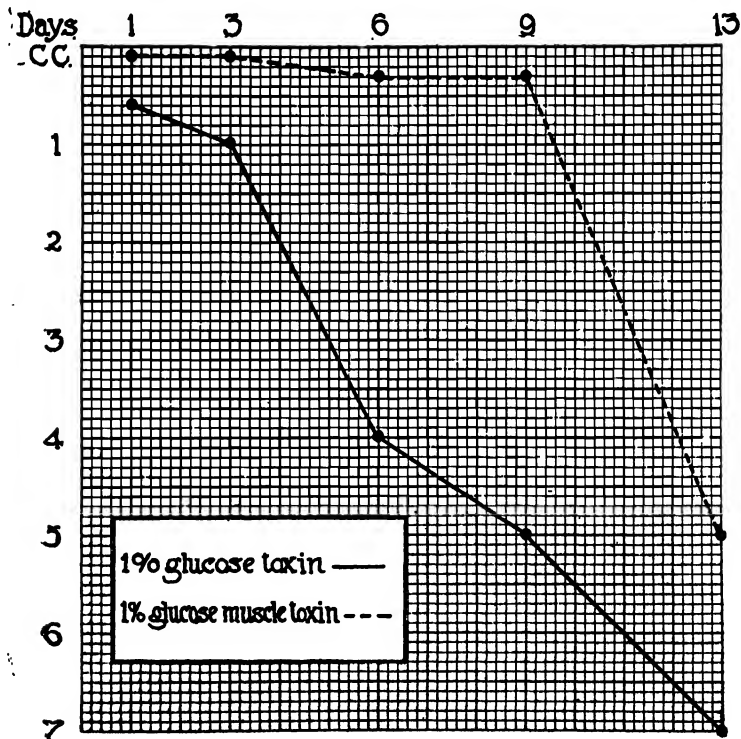
From Text-figs. 1 to 4 it is seen that the toxicity of the filtrates rapidly diminishes as the incubation time is prolonged. The toxicity



TEXT-FIG. 3. The media, 0.5 per cent glucose broth and 0.5 per cent glucose broth plus muscle. The filtrates, 0.5 per cent glucose toxin and 0.5 per cent glucose muscle toxin.

was in every instance highest at the end of the 1st day's incubation. On the 3rd day there was a material decrease in toxicity, especially in the case of the filtrates from the cultures to which muscle had not

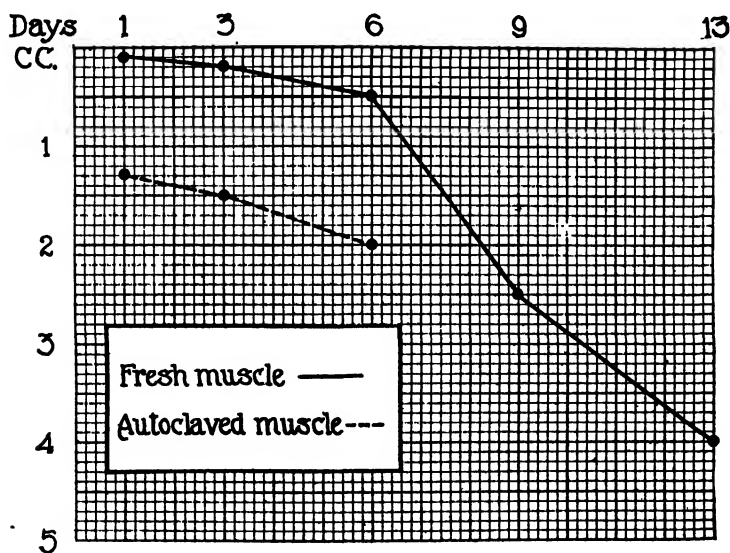
been added. A composite curve of the filtrates from the muscle medium would show that on the 6th day the toxicity had fallen almost 300 per cent. A similar curve of the filtrates from the non-muscle medium would show a decrease in toxicity of 400 per cent. A composite curve including the filtrates from both kinds of media would show a decrease in toxicity of about 1,300 per cent on the 13th



TEXT-FIG. 4. Media, 1 per cent glucose broth and 1 per cent glucose broth plus fresh muscle. Filtrates, 1 per cent glucose toxin and 1 per cent glucose muscle toxin.

day of incubation. It is evident, therefore, that prolonged incubation brings about a destruction of the toxin independently of the nature of the medium, and the most potent toxin is to be obtained at about the end of the 1st day's incubation, the optimum incubation time varying apparently from 18 to 24 hours.

The influence of the muscle upon the potency of the filtrates is very apparent. The 24 hour muscle medium filtrates were in each case about five times as toxic as the corresponding non-muscle filtrates. If the curves had been based on killing power alone, instead of on local effects and killing power, the muscle and non-muscle curves would have been still more widely separated. The toxins formed in the muscle medium seem to have a killing power not possessed by the non-muscle products. Moreover, the non-muscle filtrates lose toxicity more rapidly as the incubation time is prolonged than the



TEXT-FIG. 5. Media, 0.2 per cent glucose broth plus fresh muscle and 0.2 per cent glucose broth plus autoclaved muscle.

muscle filtrates do. The influence of the muscle in the medium is, however, largely a quantitative one. From Text-fig. 5 it is seen that autoclaved muscle will not take the place of fresh muscle.

#### *Glucose, Acidity, and Toxicity.*

It is well known that *Bacillus welchii* produces acids, chiefly butyric, under almost any condition of growth, the quantity produced depending largely upon the amount of fermentable sugars present.



Acid production being so prominent and constant, it is natural that students of this organism should have given special attention to that property. Thus, McCampbell<sup>2</sup> made many experiments on the toxic effects of *Bacillus welchii* cultures and their products and concluded that the acids are of prime importance. This conclusion was based on the observation that his neutralized cultural products were inactive, and that butyric acid in a pure state produced identical results with the acid bacillary products. Stewart and West<sup>3</sup> also state that acid by-products are responsible for all the pathologic effects of *Bacillus welchii* infections. Wright<sup>4</sup> holds similar views and has offered theoretical explanations of the way in which the acids operate in bringing about their destructive effects. In our previous paper<sup>1</sup> we have stated that neutralization with sodium hydroxide did not materially alter the pathologic effects of the cultural products, and there was no direct relation between acidity and toxicity. Further observations have been made on this point, and the results are graphically presented in Text-figs. 6 to 10.

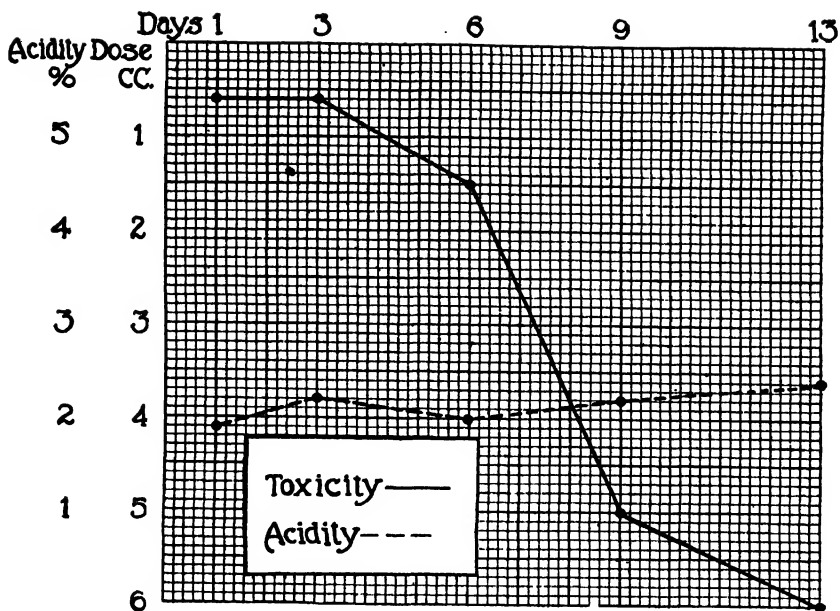
Fragments of fresh rabbit muscle were added to tubes of beef infusion broth containing varying percentages of glucose. A number of tubes of each kind of medium was inoculated with *Bacillus welchii*, a layer of oil added, and the culture tubes were exhausted in a vacuum jar and incubated in the manner already described. Filtrates were collected from a number of the tubes after from 1 to 13 days' incubation and tested for pathologic effects and acidity (Text-figs. 6 to 10).

A study of Text-figs. 6 to 10 discloses the fact that the toxicity of the filtrates is independent of the acidity. The toxic potency of the filtrates rapidly diminishes as the incubation time is prolonged, while the acidity increases or remains constant. In one instance, Text-fig. 9, there was a decrease of 1 per cent in the acidity, but the toxin lost  $\frac{3}{4}$  of its potency. When the acidity is high the filtrates show very slight toxic action. Text-fig. 10 illustrates this point. The acidity was 6.8 per cent on the 1st day, rose to 8 per cent by the 3rd day, and remained constant throughout the experiment. The toxicity curve does not represent the actual potency of the filtrate,

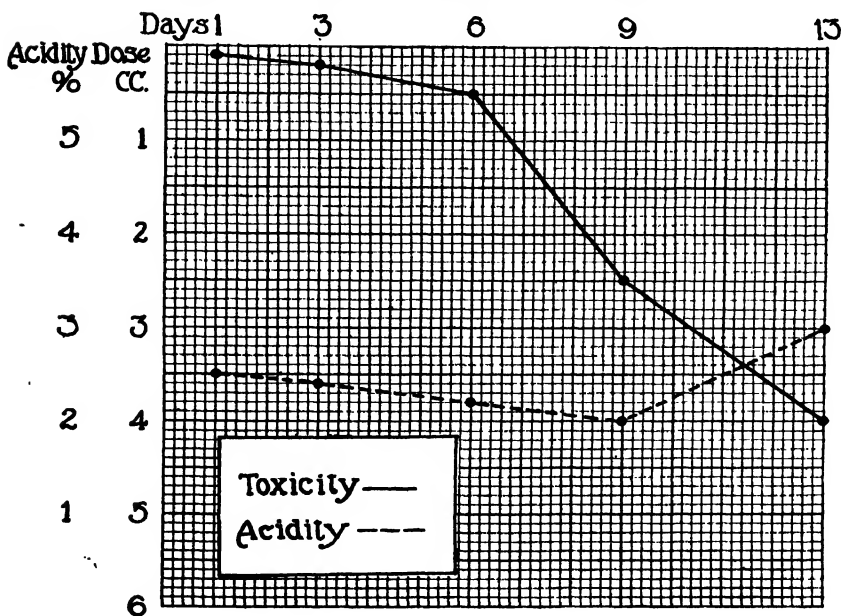
<sup>1</sup> McCampbell, E. F., *J. Infect. Dis.*, 1909, vi, 537.

<sup>2</sup> Stewart, M. W., and West, R., *J. Immunol.*, 1916, i, 189.

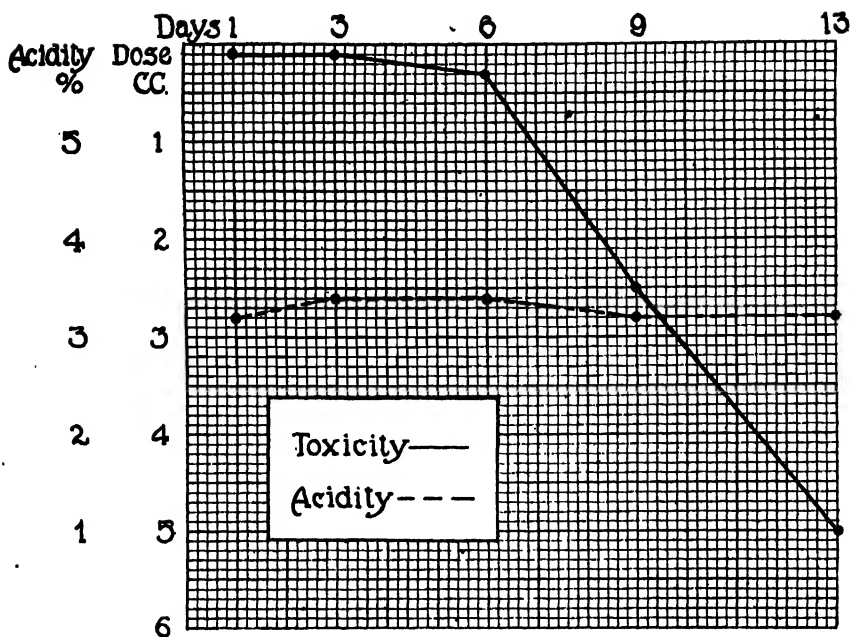
<sup>4</sup> Wright, A. E., *Proc. Roy. Soc. Med.*, 1916-17, x, Occas. Lect., 1.



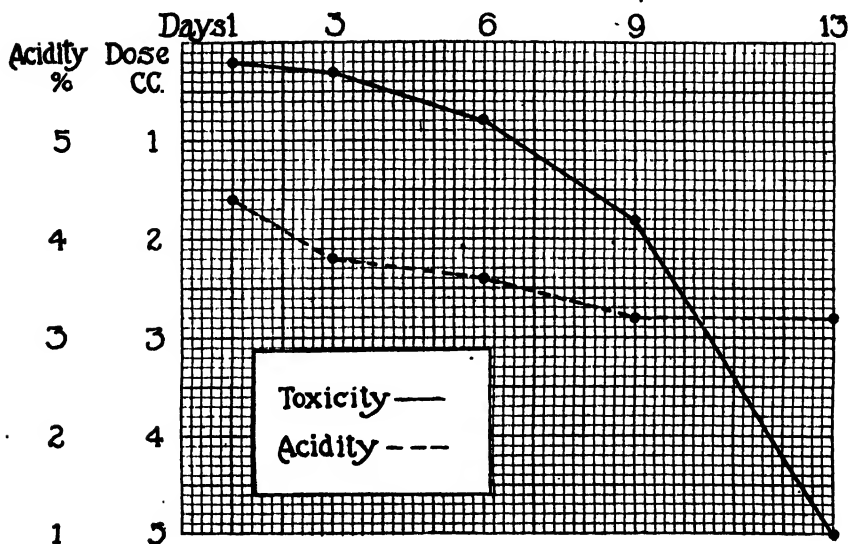
TEXT-FIG. 6. Medium, sugar-free muscle broth.



TEXT-FIG. 7. Medium, 0.2 per cent glucose muscle broth.

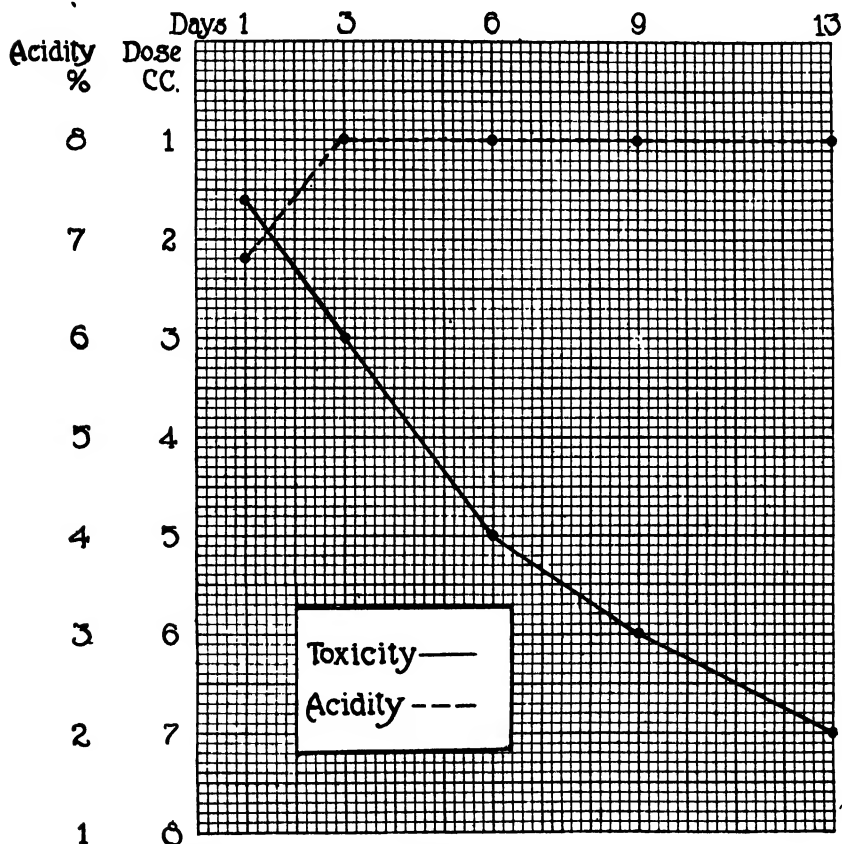


TEXT-FIG. 8. Medium, 0.5 per cent glucose muscle broth.



TEXT-FIG. 9. Medium, 1 per cent glucose muscle broth.

since the quantities used for the animal inoculations were not sufficient to cause death or definite local reactions, the curve being developed from the largest amounts inoculated at the various periods. Beginning with Text-fig. 6, there is a material increase in acidity, which reaches its highest point in Text-fig. 10, while the toxicity increases until Text-fig. 9 is reached, where an abrupt decrease is shown.



TEXT-FIG. 10. Medium, 3 per cent glucose muscle broth.

Text-figs. 6 to 10 may also be used to show the influence of glucose upon toxin production. The glucose content of the medium ranged from 0 to 3 per cent. Since muscle fragments were added to each variety of medium, the percentage of sugar was in each case higher

than is indicated. It is seen that 0.2 per cent glucose causes a material increase in toxicity (Text-figs. 6 and 7). The glucose may be increased to 1 per cent without greatly influencing the potency of the product, while more than 1 per cent has a deleterious effect. The influence of the glucose is, in all probability, non-specific, merely leading to a more luxuriant growth of the bacilli. *As a routine procedure, a 0.2 or 0.3 per cent glucose muscle broth is used for toxin production.*

### *Review of the Literature.*

In our first publication on toxin production by *Bacillus welchii* only brief consideration was given to the work of others on this subject. We propose now to cover the ground somewhat more fully.

In 1904 Kamen<sup>5</sup> reported that in gas bacillus infections in man death occurs under conditions indicating a severe intoxication. He showed also that filtrates from 8 day glucose bouillon cultures of the gas bacillus contained hemolytic substances demonstrable *in vitro*. On the other hand, 10 cc. of the same filtrates caused no lesions on subcutaneous injection in guinea pigs. The author concluded that energetic toxins were not produced in cultures, but that in all probability powerful toxins were produced in the animal body.

Passini's<sup>6</sup> publications on the subject appeared in 1905. He employed a special medium consisting of fresh beef muscle digested with trypsin and sterilized in live steam; to this glucose was added to 1 per cent or more and the air driven out by boiling; or the medium was made by heating the meat-water mixture in an autoclave under from 8 to 9 atmospheres for 1 hour. The medium was then inoculated with the bacilli and incubated for from 14 days to 1 month at 37°C. The filtrates from the cultures were found to be toxic. The toxic effects were manifested immediately on intravenous and intraperitoneal inoculation. When given subcutaneously, edema, necrosis, and sloughing followed. The toxic filtrates resisted heating to 100°C. for 15 minutes, and they were not shown to possess antigenic properties. It is also stated that the filtrates were not toxic if less than 1 per cent glucose was present.

In a general study of the intestinal flora, Metchnikoff gave considerable attention to the gas bacillus.<sup>7</sup> In his case impure cultures were evidently used since the medium was made with finely chopped beef and tap water, without any attempt at subsequent sterilization. The filtrates from the cultures were found

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<sup>5</sup> Kamen, L., *Centr. Bakteriöl., 1te Abt., Orig.*, 1904, xxxv, 554.

<sup>6</sup> Passini, F., *Wien. klin. Woch.*, 1905, xviii, 921.

<sup>7</sup> Metchnikoff, E., *Ann. Inst. Pasteur*, 1908, xxii, 951.

to be toxic, the toxicity being greatest after from 2 to 5 days' incubation. A longer incubation period caused a progressive diminution in toxicity. Heating to 100°C. did not reduce the potency of the toxin obtained; moreover, it was not tested for antigenic properties.

Schultze<sup>8</sup> showed that the disappearance of the nuclei in the organs of animals dying of gas bacillus infection was not due to gas formation, and he inferred that the phenomenon was caused by chemical toxic substances formed by the bacilli.

Korentchevsky,<sup>9</sup> working in Metchnikoff's laboratory, found that filtrates from bouillon cultures of the gas bacillus were toxic for rabbits, especially young rabbits. Symptoms of intoxication appeared in from 1 to 3 hours after intravenous and intraperitoneal injections. The chief symptoms were dyspnea, convulsive movements of the head, opisthotonos, clonic convulsions of the extremities, and paralysis. The effects of intramuscular and subcutaneous injections were not determined. Rabbits and dogs were given large quantities (20 cc.) of the filtrates *per rectum* every 2nd or 3rd day for 2½ weeks. The growth of the animals was arrested, and some lost weight. Agglutinins, precipitins, and fixatins were found in the sera of the rabbits. No mention is made of the thermostability, acidity, or antitoxin-producing properties of the filtrates.

Klose<sup>10</sup> studied 135 cases of gas phlegmon, in 39 of which the Fränkel gas bacillus (*B. welchii*) was present. The chief symptom in the cases was intoxication. Toxic substances were demonstrated in the blood sera of 5 cases of gas phlegmon in man. The clinical observations apparently led to the experimental work.

The organism used in the animal experiments was isolated from a foudroyant case of gas phlegmon in man. Toxic substances were demonstrated in the subcutaneous exudates occurring in infected guinea pigs and in Berkefeld filtrates from 5 per cent glucose broth cultures at the end of 14 days' incubation. Subcutaneous injections in guinea pigs gave rise to edema, discoloration, falling of

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<sup>8</sup> Schultze, W. H., *Virchow's Arch. path. Anat.*, 1908, cxcii, 419.

<sup>9</sup> Korentchevsky, W., *Ann. Inst. Pasteur*, 1909, xxiii, 91.

<sup>10</sup> After our first publication on *B. welchii* toxin and antitoxin appeared, our attention was called to an abstract in the *Chemical Abstracts*, July 20, 1917, of an article by F. Klose entitled: "Ueber Toxin- und Antitoxinversuche mit dem Fränkelschen Gasbrandbacillus." This abstract was made by Julian H. Lewis from an abstract which appeared in the *Chem. Zentr.*, 1916, ii, 24. The original article appeared in the *Munch. med. Woch.*, 1916, lxiii, 723. This journal could not be found in any of the libraries in New York City or elsewhere in the United States. The abstracts contained only a few summary statements without any experimental details, and no idea of the nature of the work could be formed. The original article has, however, recently come to our hands through the kindness of Dr. J. E. J. King, who returned from Germany in April after rupture of diplomatic relations between the United States and Germany. Because of this accidental circumstance, it is possible to consider the work here.

hair, necrosis, and sloughing; intraperitoneal and intravenous injections caused immediate symptoms, tremors, failure of respiration, and death. The toxic substances were quite thermostable, since 80°C. for 1 hour did not reduce their toxic effects. A horse was immunized as follows: 2,415 cc. of filtrate from 5 day 5 per cent glucose broth cultures were given intravenously during 17½ weeks and after a rest period of 6 weeks, 1,780 cc. of filtrate were given during 6½ weeks. The immune serum protected guinea pigs against three lethal doses of bacilli; 2 cc. of serum were injected at the site of infection 24 hours previously. Infections were controlled by giving the immune serum 2 hours after the guinea pig had been infected.

We have made repeated attempts to produce *Bacillus welchii* toxin artificially according to Klose's method. The filtrates were collected both after 5 days' and 14 days' incubation. Large quantities (5 cc.) of the filtrates failed to cause death or necrosis on intramuscular injection in guinea pigs and pigeons. There was, in some instances, a slight tumefaction of the tissues at the point of inoculation. In the light of these results it is somewhat difficult to explain the pathologic effects of Klose's filtrates. The neutralization and protection experiments, on the other hand, can be readily explained by the fact, observed by us, that a certain number of horses possess natural *Bacillus welchii* antitoxin. Klose did not determine the natural antitoxin content of his horse's serum.

The work of Weinberg and Séguin<sup>11</sup> was referred to in our first publication. These authors made numerous reports on the production of specific antitoxic sera for the bacillus of malignant edema (*vibrio septique*) and for *B. œdemiens* (Weinberg). They reported also that a potent specific antibacterial serum had been produced by immunizing horses with living cultures of *B. welchii* (*B. perfringens*). Frequent mention is made of a *B. perfringens* toxin, but nothing is stated concerning its preparation, or physical or biological properties, but a definite statement is made that it has not been found possible to prepare an antitoxic serum for the organism. Finally, these authors have recently reported on the clinical application of their antimicrobial serum<sup>12</sup> for *B. perfringens* (*B. welchii*) infection.

#### SUMMARY.

Twenty-two additional strains of *Bacillus welchii* have been collected from widely different sources and tested with regard to toxin

<sup>11</sup> Weinberg, M., *Proc. Roy. Soc. Med.*, 1916, ix, Occas. Lect., 119.

<sup>12</sup> Weinberg, M., and Séguin, P., *Compt. rend. Acad.*, 1917, clxv, 199.

production. Each strain produces a toxin which, on animal inoculation, gives rise to lesions comparable in every respect to those produced by the toxins previously reported on,<sup>1</sup> and each toxin was neutralized by an immune (antitoxic) serum produced with one of the former toxins. The toxins obtained from the several individual strains varied in potency, the lethal dose ranging from 0.3 to 3 cc.

Experiments have been made to determine the influence of fresh muscle and glucose on toxin production and the relation of acidity to toxicity in the filtrates. It has been found that the addition of fresh muscle to the medium increases the potency of the toxin five-fold. Autoclaved muscle is without effect. Beef infusion broth containing 0.2 to 1 per cent glucose gives a more potent product than sugar-free broth, while when higher percentages are employed the toxin production is lowered. There is no direct relation between acidity and toxicity, the most acid products manifesting little or no toxic action. In every medium used for culture the potency of the filtrates rapidly diminished after 24 hours' incubation, while the acidity increased or remained constant. The exception to this rule has been pointed out.

The most active toxin is obtained by growing a virulent strain of the bacilli in a 0.2 or 0.3 per cent glucose broth to which fragments of fresh muscle have been added, and collecting the filtrate after from 18 to 24 hours' incubation.

A review of the literature on the pathogenic effects and toxic products of *Bacillus welchii* and on the results of immunization of animals with the bacilli or toxic products does not indicate that the exotoxic nature of *Bacillus welchii* had been previously determined or an antitoxic serum in the true sense produced.

The antitoxin for *Bacillus welchii* toxin can apparently be prepared from a single strain of the organism which yields under the conditions described a high titer of toxin, and this antitoxin can be employed to combat infection with or prevent infection by any strain whatever of the bacillus.





## A STUDY OF MITOCHONDRIA IN EXPERIMENTAL POLIOMYELITIS.

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### INTRODUCTION.

Although many observations have been made on mitochondria in normal tissues, both adult and embryonic, the study of these structures in pathological material has been relatively limited and the results in some cases have been conflicting. The information regarding this type of cell granule has been summarized by Cowdry,<sup>1</sup> but in view of the fact that several observers have recorded changes in mitochondria, often occurring quite early in certain lesions,<sup>2-13</sup> it seemed possible that some such results might be obtained in the case of poliomyelitis, which would throw light on the pathology of that condition.

Spinal ganglia were employed for the study, because they show typical lesions in monkeys dying of experimental poliomyelitis, and because, of the structures showing these lesions, they could be most

<sup>1</sup> Cowdry, E. V., *Am. J. Anat.*, 1916, xix, 423.

<sup>2</sup> Barrett, J. O., *Quart. J. Micr. Sc.*, 1913, lviii, 214.

<sup>3</sup> Beckton, H., *Arch. Middlesex Hosp.*, 1909, xv, 182.

<sup>4</sup> Beckton, H., and Russ, S., *Arch. Middlesex Hosp.*, 1911, xxiii, 59.

<sup>5</sup> Bensley, R. R., *Tr. Chicago Path. Soc.*, 1909-12, viii, 78.

<sup>6</sup> Ciaccio, C., and Scaglione, S., *Beitr. path. Anat. u. allg. Path.*, 1913, lv, 131.

<sup>7</sup> Goetsch, E., *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 29.

<sup>8</sup> Homans, J., *J. Med. Research*, 1915-16, xxxiii, 1.

<sup>9</sup> Regaud, C., and Favre, M., *Compt. rend. Soc. biol.*, 1911, lxviii, 658.

<sup>10</sup> Regaud and Favre, *Compt. rend. Soc. biol.*, 1912, lxix, 328.

<sup>11</sup> Romes, B., *Anat. Anz.*, 1913-14, xlv, 1.

<sup>12</sup> Scott, W. J. M., *Am. J. Anat.*, 1916, xx, 237.

<sup>13</sup> Strongman, B. T., *Anat. Rec.*, 1917, xii, 167.

successfully obtained and fixed. Cowdry<sup>14</sup> has given a full account of the mitochondria occurring in normal ganglion cells of vertebrates.

#### EXPERIMENTAL.

In a few instances injection fixation was employed. Here the method described by Cowdry<sup>1</sup> was used with the following variations. The formalin bichromate mixture was more successful when used in half rather than in full strength. 2 to 3 feet of gravity pressure of injection fluid were found to give less edema and distortion of the tissues than the higher pressure (4 to 6 feet), so that while the saline infusion was given at the higher pressure for the first 5 to 10 minutes to insure complete washing out of clots, the lower pressure was maintained throughout the remainder of the period. The femoral vein of one side was cut, rather than the vena cava, as the latter gave too rapid an outflow of fluid. The cardiac, mesenteric, and opposite iliac arteries were clamped. The injection of fixative was continued for 1 hour and the best results were obtained when the animal was kept lying on the board back down, for  $\frac{1}{2}$  hour longer before autopsy.<sup>15</sup> Injection was less successful in poliomyelitic monkeys and in the lumbar region of operated rabbits than in normal animals, possibly owing to vascular lesions in the former.

The ganglia, however, were quite as well fixed by simple immersion in fixing fluid. The animals were usually chloroformed and the ganglia taken at once. In a few cases the animals died and were autopsied within 2 hours of death. The specimens were placed in formalin bichromate mixture (3 per cent potassium bichromate 4 parts, neutral formalin 1 part, and water 5 parts) for 4 days and then transferred to half strength ( $1\frac{1}{2}$  per cent) potassium bichromate for 5 days. This procedure was found to give better results than the solution ordinarily used. Full strength solutions and the acetic-osmic-bichromate mixture<sup>16</sup> (2.5 per cent bichromate 8 cc., 2 per cent osmic acid 2 cc., and glacial acetic acid 1 drop) in full strength or diluted to half strength were apt to give good fixation only in the

<sup>14</sup> Cowdry, *Am. J. Anat.*, 1914-15, xvii, 1.

<sup>15</sup> Schirokogoroff, J. J., *Anat. Anz.*, 1913, xliii, 522.

<sup>16</sup> Bensley, *Am. J. Anat.*, 1911-12, xii, 297.

case of superficial cells. The osmic acid mixture also darkened the whole section to such an extent that it dimmed the contrast between mitochondria and small Nissl bodies.

At first the use of chloroform in embedding was tried, but in many cases the mitochondria disappeared under this treatment. In one case the tissue was first embedded by the xylol method and sections were cut, then reembedded by the chloroform method.<sup>17</sup> The former sections showed mitochondria, but none was present in the latter. This may have been due to rehandling, however. Embedding was done as described by Cowdry,<sup>14</sup> except that absolute alcohol-xylol for 1 hour, xylol 1 hour, paraffin 3 hours, was found to be sufficient and less liable to destroy the mitochondria. Both the acid fuchsin-methyl green, and the iron-alum-hematoxylin (Regaud and Favre<sup>9</sup>) methods were used for staining.

*Rabbits.*—In order to compare cell changes in another form of paralysis a series of rabbits was used, in some of which ischemic paralysis of the hind legs was produced by the Stenson operation (Fredericq,<sup>18</sup> Ehrlich and Brieger<sup>19</sup>). The animals were etherized. An area about 3 inches wide, extending from ensiform process to symphysis pubis, was shaved and cleaned with alcohol. Aseptic technique was employed. An incision was made in the midline, and the intestines were covered with cloths wet with warm saline. The abdominal aorta was exposed and a soft bulldog clamp placed on it about  $\frac{1}{2}$  inch below the renal arteries, the effectiveness of the clamp being tested by palpation of the vessel below it. The intestines were replaced and the animal was kept under light ether anesthesia, the clamp being left in place for  $\frac{1}{2}$  or  $\frac{3}{4}$  hour. The longer period was found to give certain results while the former failed in some cases to give paralysis. Care was taken to keep the animal warm during this period. At the end of the period the clamp was removed, the abdominal wall sewed with silk, and the animal allowed to recover.

In almost every instance, flaccid paralysis of the hind legs was evident as soon as the animal recovered from the ether. In two rabbits

<sup>17</sup> Mallory, F. B., and Wright, J. H., *Pathological technique*, Philadelphia and London, 6th edition, 1915, 284.

<sup>18</sup> Fredericq, L., *Arch. biol.*, 1890, x, 131.

<sup>19</sup> Ehrlich and Brieger, *Z. klin. Med.*, 1884, vii, Supplement, 155.

in which the shorter compressions were used, the hind legs were spastic, with convulsive twitchings, which gradually disappeared, the animal recovering the full use of the legs.

The animals were chloroformed at various periods after the ligation and fixed by the injection method. The lumbar region of the cord did not always take the fixative so well as did the cervical region, or the lumbar region of normal animals, and in those animals killed 12 or more hours after the clamping, the cords remained extremely soft, and were uncolored by the chromate and very difficult to handle.

*Material.*—A series of ganglia was obtained from fourteen monkeys with experimental poliomyelitis.<sup>20</sup> Six were either in the preparalytic stage, without definite lesions in the ganglia, though having shown such symptoms as irritability, etc., or if they showed paralysis, the particular ganglia used failed to show typical lesions. The remaining ten, taken from the 1st to the 7th day after the onset of paralysis of some muscle group had been noted, all showed typical lesions of the cord and ganglia, including those used for mitochondria, as shown by examination in gross or of sections stained by hematoxylin and eosin.

Five monkeys were used as controls. Two of these showed lesions of tuberculosis at autopsy. One was an apparently normal monkey which died while being etherized, and the viscera showed no gross abnormalities. The other two monkeys had received poliomyelitis virus intranasally but showed no symptoms either during life or post mortem.

*Results.*—The ganglia from the five control monkeys and those from the six poliomyelitic monkeys presenting no lesions in the ganglia used showed mitochondria similar to those described by Cowdry in the normal animal. Great variations were found in the number of mitochondria and the intensity with which they took the stain, and several showed many cells in the chromatophilic state.<sup>21</sup> In one, considerable postmortem degeneration had occurred.<sup>22</sup> One showed a large amount of typical lipid. All, however, were cells similar to those described by Cowdry as normal cells. This was true also

<sup>20</sup> Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

<sup>21</sup> Cowdry, Contributions to embryology, *Carnegie Institution of Washington, Publication No. 224*, 1916, Contribution No. xi.

<sup>22</sup> Ciaccio, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 721.

of the ganglion cells from the normal rabbits and those from the cervical region of the operated rabbits. In these rabbit sections, no chromatophil cells were found.

In the tissues from the poliomyelitic animals also, many cells were normal in appearance. In many, moreover, the mitochondria appeared to be even more clearly shown than in normal cells. This appearance may have been due to disappearance of Nissl substance, which was reduced in these cells. In normal cells it was often hard to differentiate between mitochondria and small Nissl bodies as the granules were of nearly the same size and in some preparations tended to take the fuchsin stain. Many cells contained much particulate lipid and many were in the chromatophilic state, but in these particulars they did not seem to exceed normal limits.

In the cells which showed marked neurophagocytosis, mitochondria-like threads could often be seen, even though only a small remnant of protoplasm remained. They appeared as minute reddish threads or dots, or larger masses, even to fairly large rods, lying in the usual bluish background and in spaces between the invading cells. They did not have the globular shape characteristic of lipid, and they seemed to have some of the chemical reactions of the mitochondria, for they were not found in slides in which the mitochondria had been lost through poor fixation. In cells in which the destruction had been less complete, typical mitochondria were found to persist in an apparently normal ratio to cell substance.

A similar, though less marked persistence of mitochondria was also noted in the lumbar ganglia of the rabbits. 1 hour after the production of the anemia, a few darkly staining cells were found, showing a few reddish threads against a dark purplish background. At 7 hours almost all the cells had this chromatophilic tendency, a few still showing the reddish threads, but in the majority there was only a shrunken, irregularly stained protoplasm. At 12 hours, the position of a few remaining cells was indicated by dark, indefinite spots.

#### DISCUSSION.

If these red-staining threads occurring in the invaded cells in poliomyelitis are mitochondria, the mitochondria in this condition

at least outlast any other cell structure now recognized. This is remarkable in view of their usual tendency to disappear under slight changes, such as acidity or temperature. Since the stain is not absolutely specific, and we lack means at the present time of differentiating mitochondria with certainty from other lipoidal structures, it may be that these threads, or rods, are merely part of a coagulum of some different nature. Yet, since typical mitochondria persist after the disappearance of typical Nissl substance, and various gradations can be traced up to the stage of almost total replacement of the original ganglion cell, it would seem safe to call these mitochondria under the present use of the term.

It would be desirable to have some means of quantitative estimation of the mitochondria for these purposes, but the one so far described (Thurlow<sup>23</sup>) does not prove applicable to the rod-like forms.

#### CONCLUSION.

Typical mitochondria can be found in the spinal ganglion cells of monkeys with experimental poliomyelitis, even when typical Nissl substance has disappeared, and mitochondria-like structures are found in the remaining protoplasm in the latest stage of neurophagocytosis.

<sup>23</sup> Thurlow, M., Contributions to embryology, *Carnegie Institution of Washington, Publication No. 226*, 1917, Contribution No. xvi.

## THE SOLVENT ACTION OF ANTISEPTICS ON NECROTIC TISSUE.

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PLATE 5.

(Received for publication, October 1, 1917.)

The recent interest in the chemical sterilization of wounds has led to the introduction of numerous new antiseptics, each of which has in turn been advocated because of some advantage, real or apparent. For many of these compounds, claims have been made which are not always confirmed by carefully controlled experiments. Carrel and Dehelly<sup>1</sup> emphasize that, for the removal of the necrotic tissue that remains after mechanical cleansing, Dakin's hypochlorite solution is the antiseptic of choice because of its solvent action on devitalized tissue, and Dakin and Dunham<sup>2</sup> also recognize the value of the hypochlorite solution for this purpose.

Dakin's solution was shown by Fiessinger and his coworkers<sup>3</sup> to have a disintegrating action on pus cells. Rous and Jones<sup>4</sup> have shown that intact leukocytes may protect virulent bacteria which they have phagocytized from the action of antiseptics, and that subsequently these bacteria may proliferate under suitable conditions. Dakin's solution, by its solvent action on these leukocytes, minimizes the danger of reinfection of the wound from this source. Because of this action on necrotic tissue, pus, and serum clot, Carrel and Dehelly recommend Dakin's hypochlorite solution for the sterili-

<sup>1</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 150, 192.

<sup>2</sup> Dakin, H. D., and Dunham, E. K., *A handbook on antiseptics*, New York, 1917, 14.

<sup>3</sup> Fiessinger, N., Moiroud, P., Guillaumin, C. O., and Vienne, G., *Ann. med.*, 1916, iii, 133.

<sup>4</sup> Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.



zation of infected wounds.<sup>5</sup> Bashford<sup>6</sup> has demonstrated the ability of Dakin's solution in high dilution to erode the tissues of the tadpole's abdomen. He showed also that this occurred only after the circulation to the part had been interrupted for some time, due to the death of the organism.

As this erosive action of Dakin's solution is an important factor, the following experiments were planned to compare its solvent action with that of certain other chlorinated antiseptics. Fiessinger and his coworkers<sup>8</sup> concluded that the essential factor in the solvent action of the hypochlorites is their alkalinity. Our experiments were therefore designed to determine the importance of three factors: the alkalinity, the nature of the chlorinated antiseptic employed, and the chlorine concentration of the latter.

#### *Method.*

The solvent action of the various substances employed was tested by adding 50 cc. of each solution to 5 cc. of an emulsion of macerated liver tissue in a 100 cc. bottle. The mixture was thoroughly shaken every half hour for 2 hours. A 15 cc. portion was then removed to a centrifuge tube and in each case centrifuged at the same high speed for 5 minutes. The volume in cubic centimeters of the sediment thrown down was measured. The solvent action was shown by diminution of the amount of sediment compared with that obtained from inert solutions such as water or normal saline solution.

The liver emulsion was prepared in Experiment 1 from rabbit liver, in the other experiments from cat liver. In Experiments 1, 2, 3, and 4 the liver was purposely infected by handling, placed in the incubator at 37°C. until thoroughly necrotic, cut into small pieces, suspended in saline solution, shaken in a bottle with broken glass to emulsify it, and strained through a single layer of gauze. In Experiment 5 cat liver was similarly emulsified and used after 12 hours' preservation in the ice box. The solutions employed were prepared as follows:

<sup>5</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 109.

<sup>6</sup> Bashford, E. F., *Lancet*, 1917, cxci, 595.

*Control Solutions.*—Neutral: water and normal saline solution. Weakly alkaline: a solution of sodium carbonate, 1 gm., and sodium bicarbonate, 17 gm. per liter of water; this solution has approximately the alkalinity of a properly prepared Dakin's solution. Strongly alkaline: 0.1 N sodium hydroxide.

*Chloramine-T Solutions.*—Chloramine-T solutions were prepared by dissolving the required amount of chlorazene<sup>7</sup> in the appropriate control solution to obtain neutral, weakly alkaline, or strongly alkaline chloramine solutions.

*Hypochlorite Solutions.*—Weakly alkaline: ordinary Dakin's solution prepared either from bleaching powder or by the action of liquid chlorine on sodium carbonate solution, in either case with careful control of the degree of alkalinity as well as of the hypochlorite content. Neutral: chlorine gas passed through sodium carbonate solution, 28 gm. per liter, until the solution just ceased to give a flash of pink upon the addition of alcoholic solution of phenolphthalein, 1 per cent; the hypochlorite content was determined by titration, and the solution diluted with water to the desired strength. This solution was always prepared immediately before use, as a considerable proportion of the total hypochlorite is present as hypochlorous acid, and the decomposition of the solution is very rapid (Cullen and Austin<sup>8</sup>). Strongly alkaline: A double strength neutral solution of hypochlorite was prepared as just described and immediately added to an equal volume of 0.2 N sodium hydroxide solution.

*Chlorinated Oils.*—Paraffin oil and eucalyptol were mixed in equal parts, and the same oils chlorinated according to Dakin's method.<sup>9</sup>

*Dichloramine-T.*—A 15 per cent solution was made in chlorinated eucalyptol and then mixed with an equal volume of chlorinated paraffin oil. In the experiments in which the oils were used (Experiment 1, Tubes 5, 6, and 7; Experiment 2, Tubes 4, 5, and 6; Experiment 3, Tube 9), except in Experiment 3, Tube 10, 5 cc. of liver emulsion were added to 50 cc. of water or of one of the control solutions, and 15 cc. of the oil used were superimposed upon this mixture; the mixture was well shaken every half hour with a rotary motion.

<sup>7</sup> Prepared by the Abbott Laboratories, New York.

<sup>8</sup> Personal communication.

<sup>9</sup> Dakin, H. D., *Brit. Med. J.*, 1915, ii, 318.

At the end of 2 hours, after again being shaken, the oil was allowed to separate from the aqueous suspension and 15 cc. portions were immediately removed from the latter for centrifuging. In Tube 10 of Experiment 3, the 5 cc. of liver emulsion were introduced into 50 cc. of the oil without water and shaken continuously for 2 hours.

The sodium hypochlorite equivalent of the hypochlorite and other chloramine solutions was determined by the addition of potassium iodide solution and glacial acetic acid to 10 cc. samples and titration of the iodine liberated with 0.1 N sodium thiosulfate.

### RESULTS.

In Experiment 1, Table I, Solutions 8 and 9 being taken as controls, no solvent effect was noted after the action of chloramine-T solution or of the chlorinated oils, with or without dichloramine-T. Dakin's solution, on the other hand, had a marked solvent action, which was apparent upon inspection of the bottles, even in the first 15 minutes. This was accompanied by pronounced bleaching of the emulsion. A still more rapid and marked action, similar in character, was obtained from the strongly alkaline hypochlorite. Figs. 1 and 2 show the results of this experiment at the end of 2 hours. The reaction of the

TABLE I.  
*Experiment 1.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral chloramine-T, 0.5 per cent, equivalent to sodium hypochlorite, 0.12 per cent.....	0.30
2	Neutral chloramine-T, 0.2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.25
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's solution)).....	0.05
4	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent).	0.02
5	Water + paraffin oil and eucalyptol.*.....	0.27
6	" + chlorinated paraffin oil and eucalyptol.*.....	0.28
7	" + " " " " " + dichloramine-T, 7.5 per cent.....	0.27
8	Salt solution.....	0.28
9	Water.....	0.27

\* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil.

solutions used, however, varied, as well as the nature of the antiseptic substance. Experiment 2 was therefore performed with solutions of approximately the same reaction (Table II).

The results of this experiment confirm those observed in Experiment 1. Chloramine-T and dichloramine-T were without solvent action, whereas Dakin's hypochlorite gave marked solution. In Experiment 3, Table III, the effect of diminishing the concentration of the antiseptic in a solution of the same reaction was tested. Both weakly alkaline and strongly alkaline solutions were employed.

TABLE II.

*Experiment 2.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Weakly alkaline carbonate-bicarbonate solution.....	1.27
2	“ “ “ “ + chloramine-T, 2 per cent.....	1.24
3	Weakly alkaline hypochlorite, stock Dakin's solution (sodium hypochlorite, 0.5 per cent) .....	0.08
4	Weakly alkaline carbonate-bicarbonate solution + paraffin oil and eucalyptol.*.....	1.23
5	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol.*.....	1.24
6	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.....	1.32

\* 5 cc. of liver emulsion in 50 cc. of carbonate-bicarbonate solution, overlaid with 15 cc. of oil.

This experiment showed a loss of the solvent action in weakly alkaline hypochlorite solutions, occurring suddenly between 0.2 and 0.3 per cent sodium hypochlorite concentration. In the strongly alkaline solutions the solvent action was marked, even at the lowest hypochlorite concentration employed. This experiment, taken in conjunction with the well known rapid drop in the sodium hypochlorite titer of Dakin's solution in contact with tissues, indicates that any solvent action resulting from its application clinically may be expected to occur in the first few minutes and emphasizes the importance of frequent flushing of wounds with the solution.

In order to distinguish between the effects of alkalinity and of hypochlorite concentration, Experiment 4 was performed (Table IV). The neutral hypochlorite solutions were prepared as described above. For the neutral control, saline solution was employed. The weakly alkaline hypochlorite solutions in the column headed "Weakly alkaline due to hypochlorite" were prepared as already described, except that the chlorine gas was passed into sodium hydroxide solution instead of into sodium carbonate, thus producing a solution of which

TABLE III.

*Experiment 3.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Water.....	0.25
2	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.22
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)...	0.01
4	" " " " " 0.4 " " ....	0.01
5	" " " " " 0.3 " " ....	0.02
6	" " " " " 0.2 " " ....	0.22
7	Strongly " " " " " 0.5 " " ....	Tr.
8	" " " " " 0.2 " " ....	0.08
9	Water + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.....	0.25
10	Chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent, without water.†.....	0.22

\* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil. Shaker continuously for 2 hours.

† 5 cc. of liver emulsion in 50 cc. of oil. Shaken continuously for 2 hours.

the alkalinity was due almost entirely to sodium hypochlorite, and which was without buffer substances. The hypochlorite solution in the column headed "Weakly alkaline due to carbonate-bicarbonate" and the strongly alkaline solution were prepared exactly as described above. Table IV shows that in the control solutions solvent action occurred only in the strongly alkaline solution. A somewhat more marked solvent action was obtained when hypochlorite, even in the low concentration of 0.1 per cent, was added to the alkali. No solvent action was obtained in the weakly alkaline control, as compared

with the neutral control of normal saline solution. When hypochlorite was added to the weakly alkaline carbonate-bicarbonate solution, or when a weakly alkaline solution of sodium hypochlorite without carbonates was prepared, no solvent action was present at a concentration of 0.1 per cent, but it was marked at a concentration of 0.2 per cent. The change in solvent action resulting from small variations in hypochlorite concentration at about 0.2 per cent was striking, confirming the results of Experiment 3. The absence of solvent action of Dakin's solution below a hypochlorite concentration of 1:500 contrasts sharply with the marked bactericidal action of the same solution in serum to 1:1,500, and in water to 1:500,000 on *Staphylococcus aureus*.<sup>10</sup> In neutral solution at a hypochlorite concentration of 0.2 per cent, solvent action was very slight. At 0.3 per cent it was moderate, and at 0.5 per cent marked.

TABLE IV.

*Experiment 4.*

Tube No.	Hypochlorite concentration of solutions.	Reaction of solutions.			
		Neutral.	Weakly alkaline.		Strongly alkaline.
			Due to carbonate-bicarbonate.	Due to hypochlorite.	
		Sediment.	Sediment.	Sediment.	Sediment.
	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
1	0.5	0.01	0.01	0.01	Tr.
2	0.3	0.04	0.01	0.01	"
3	0.2	0.10	0.01	0.01	"
4	0.1	0.14	0.13	0.12	"
5	Control.	0.12	0.12		0.04

Experiment 5 serves as a final control of the solvent action of alkali alone, of chloramine-T added to neutral, weakly alkaline, and strongly alkaline solutions, and of hypochlorite solutions of the three grades of alkalinity. It is clear that chloramine-T, even in a 2 per cent solution, has no solvent action except that due to the alkalinity of the solution in which it is dissolved, and that therefore it is without this

<sup>10</sup> Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

action in the grade of alkalinity permissible for clinical use. On the other hand, 0.5 per cent neutral sodium hypochlorite-hypochlorous acid solution (Tube 7) has a marked solvent effect, which must be attributed to the action of the chlorine unaided by alkali. Fig. 3 shows the results of Experiment 5 (Table V) at the end of 2 hours.

TABLE V.  
*Experiment 5.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral control (salt solution).....	0.25
2	Weakly alkaline control (carbonate-bicarbonate).....	0.24
3	Strongly " " (0.1 N sodium hydroxide).....	Tr.
4	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.26
5	Weakly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.24
6	Strongly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	Tr.
7	Neutral hypochlorite (sodium hypochlorite, 0.5 per cent).....	"
8	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's)).....	"
9	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)...	"

Experiments upon leukocytes, erythrocytes, and plasma clot in the various solutions employed in the five experiments described gave results practically identical with those obtained with liver emulsion. When, however, discs of blood clot were employed, solvent action could not be demonstrated except, possibly, to a slight degree in the strongly alkaline solutions. Blood clot is the most resistant of the substances studied against the solvent action of the solutions used.

#### DISCUSSION.

From the results recorded above, it seems justifiable to lay considerable stress on the relatively great solvent action of Dakin's hypochlorite solution as contrasted with the more recent and more stable chloramines of Dakin. It also seems probable that to its greater ability to dissolve necrotic tissue, plasma clot, and leukocytes

it owes its chief claim to preference over the chloramines in the treatment of infected wounds. Curves shown by Carrel and Dehelly<sup>11</sup> demonstrate the relative ease with which this solution will sterilize grossly infected wounds in the initial presence of much necrotic tissue and pus.

The results of our experiments show that the solvent action of Dakin's hypochlorite solution in the degree of alkalinity used clinically is due primarily to its hypochlorite content. The slight alkalinity of Dakin's solution, while in itself without solvent action, does, however, increase the effectiveness of the hypochlorite. We are compelled to differ, therefore, from Fiessinger and his coworkers,<sup>3</sup> who attributed this action of the hypochlorite solutions to their alkalinity. In our weakly alkaline solutions, the solvent action of the hypochlorite solution ceased abruptly at about 0.2 per cent sodium hypochlorite concentration. This phenomenon occurs at a lower hypochlorite concentration as the reaction of the solution becomes more alkaline and *vice versa*. Even in neutral solutions, marked solvent action occurs at a hypochlorite-hypochlorous acid concentration of 0.5 per cent. A solution the alkalinity of which is equal to 0.1 N sodium hydroxide exerts a solvent action in the absence of any other factor. Such a solution, however, is not available for clinical use because of its irritating properties.

Chloramine-T failed in these experiments to exhibit any solvent action not explicable as an effect of the alkalinity of the solution in which it was dissolved, and dichloramine-T was also wholly without solvent action. The results of our experimental studies do not, therefore, support the clinical observations of Dakin and his associates,<sup>12</sup> who assert that "the chlorin in dichloramin-T, as in the hypochlorites, has the power of dissolving dead tissues," or similar conclusions reached by Sweet,<sup>13</sup> who states: "The dichloramin-T also possesses to a marked degree the characteristic power of the chlorin solutions in aiding the digestion and removal of necrotic, sloughing

<sup>11</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 162, 170.

<sup>12</sup> Dakin, H. D., Lee, W. E., Sweet, J. E., Hendrik, B. M., and Le Conte, R. G., *J. Am. Med. Assn.*, 1917, lxi, 30.

<sup>13</sup> Sweet, J. E., *J. Am. Med. Assn.*, 1917, lxi, 1076.



tissues. The new solution seems more effective in cleaning up sloughing tissue than the older chlorin compounds." It seems probable that the greater solvent action of hypochlorite solution, as contrasted with the chloramines, is related to the greater instability of the former. We have been unable to demonstrate a solvent action on blood clot from any of the solutions of a reaction available for clinical use.

#### CONCLUSIONS.

1. Dakin's hypochlorite solution has the power of dissolving necrotic tissue, pus, and plasma clot in the concentration and reaction used clinically.

2. Chloramine-T and dichloramine-T do not exhibit this action.

3. The solvent action of Dakin's hypochlorite solution of the degree of alkalinity used clinically is due primarily to its hypochlorite content, but its slight alkalinity, while in itself without solvent action, enhances the effectiveness of the hypochlorite.

4. In the degree of alkalinity used clinically, the solvent action of hypochlorite is absent below about 0.2 per cent sodium hypochlorite concentration.

5. The hypochlorite concentration at which the solvent action ceases is lower the more alkaline the solution, and *vice versa*.

6. None of the antiseptics studied had demonstrable solvent action on blood clot.

#### EXPLANATION OF PLATE 5.

FIG. 1. Photograph of bottles in Experiment 1 (Table I).

FIG. 2. Photograph of centrifuged samples from Experiment 1 (Table I).

FIG. 3. Photograph of centrifuged samples from Experiment 5 (Table V).

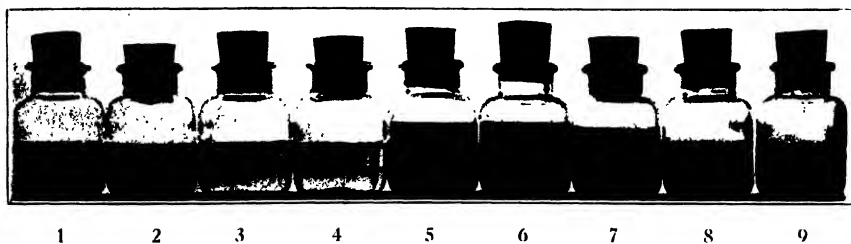


FIG. 1.

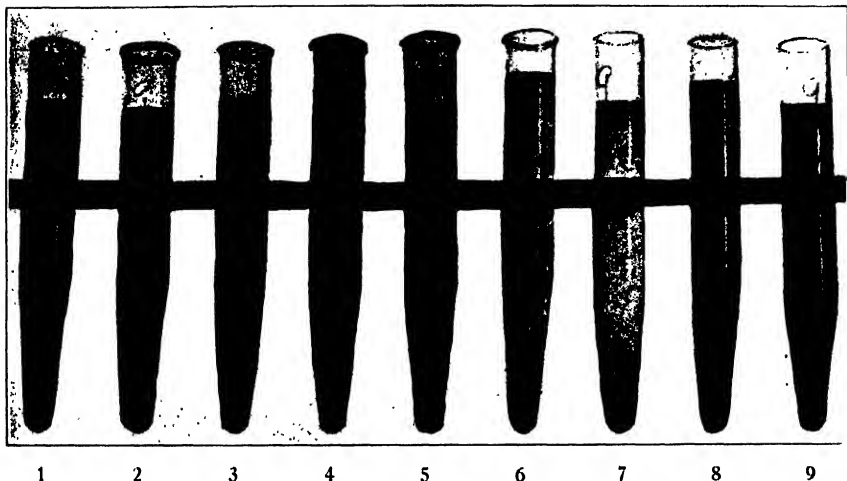


FIG. 2.

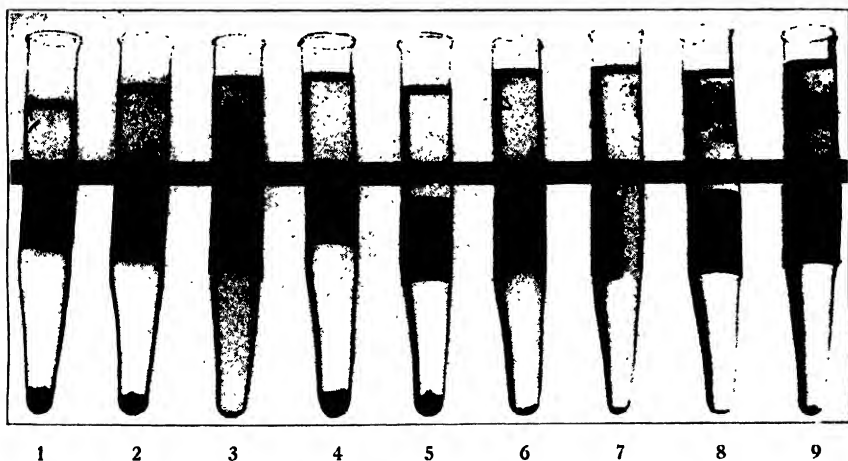


FIG. 3.

(Taylor and Austin: Solvent action of antiseptics.)



## RAPID METHOD FOR IDENTIFICATION AND ISOLATION OF MENINGOCOCCI FROM THE NASOPHARYNX.

By PETER K. OLITSKY, M.D.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

There is no subject in practical bacteriology being more actively pursued than that of the identification of the meningococcus in plate cultures prepared from the nasopharyngeal mucous membrane. This situation has been brought about through the assembling of the National Army and the National Guard, and the appearance of epidemic meningitis and meningococcus carriers in some of the Army camps. Since the detection and the segregation of the carriers constitute one measure of control of the disease, it is in the highest degree important to reduce to the minimum the time and work incident to their discovery. In this way the separation of the men from their commands is reduced, and the work of the hospital laboratories lightened. The method given here is offered as serving to save time by simplifying the identification of meningococci in mixed cultures in Petri plates. The method proposed employs a fluid medium which serves to eliminate other organisms resembling the meningococcus in about twelve hours and to reduce the full time required to identify the latter by at least one day.

### *Method.*

To 1 per cent. glucose broth (made from veal infusion and having an acidity of from 0.5 to 0.7 + phenolphthalein) is added 5 per cent. of unheated, sterile, clear normal horse serum. This medium is then distributed in small tubes (from 8 to 10 mm. diameter, and 9 cm. length), 1 c.c. being placed in each tube.

Suspicious colonies on the plate cultures are fished and seeded, a colony to each tube.

These small tubes are then incubated twelve hours (or overnight, if more convenient). At the expiration of this time, they will show

the distinctive characters of the organism in question, and at this early period "negatives" may be determined.

The bacteria that complicate the isolation and identification of the meningococcus on plate culture are the *Micrococcus flavus*, *crassus*, *pharyngis-siccus* and *catarrhalis*; the *B. influenzae*, and an unclassified gram-positive bacillus; and occasionally, in the hands of beginners, the staphylococci and streptococci.

Owing to the presence of normal horse serum in the medium, practically a normal horse serum control is at hand, so that the *M. flavus*, *crassus* and *pharyngis-siccus*, and the unclassified gram-positive bacillus will show firm agglutination. While the bacillus culture may show slight turbidity over an agglutinated sediment, the diplococci cultures show clear supernatant fluid over agglutinated masses of those organisms. As hemoglobin is absent, *B. influenzae* fails to grow. *M. catarrhalis* grows with a dense turbidity, and often shows a pellicle on the surface. The gram-positive staphylococci grow also with a dense turbidity, and show agglutinated masses in the sediment and often a surface pellicle as well. The streptococci grow with clear or turbid supernatant fluid, but show an agglutinated sediment.

The meningococci, on the other hand, grow in a characteristic manner. The fluid becomes faintly turbid, and a slight sediment forms; but, and this is all important, the sediment emulsifies uniformly when the tube is shaken.

The cultures in the fluid medium are divided into two sets by simple inspection. One set is readily excluded from being meningococci on the basis of the characters described above. The other set, which exhibits the qualities of the growth of the meningococcus, is regarded as suspicious, and to each of the tubes is added 0.1 c.c. of a 1:10 dilution in 0.85 per cent. saline solution of a high-titer polyvalent antimeningococcic serum. The tubes are then incubated in a water-bath (not in an incubator) at from 37° to 38° C. for two hours.

The reading of the tubes is definite and distinct. Those containing meningococci exhibit distinct agglutination; those containing other organisms remain unchanged. The readings are checked by means of films stained by Gram's method. All the tubes recorded

as positive will show agglutinated masses of gram-negative diplococci of typical meningococcus morphology.

From the tubes containing the agglutinated meningococci, transplants on solid mediums may be prepared for further identification or for stock cultures. The last procedure is not necessary in order to detect or exclude the suspected meningococcus carriers.

### *Advantages of the Method.*

1. The sparing of culture medium: hence more colonies from a given plate will or may be investigated.

2. The rapid growth of organisms in the small volume of medium, thus permitting the discrimination of negative and suspicious growth in about twelve hours.

3. The use of normal horse serum for enriching the medium and for the elimination of a variety of gram-negative micrococci that are subject to nonspecific agglutination. Among the latter is especially *M. flavus*.

4. The sharpness and rapidity of the reaction of agglutination produced by the polyvalent antimeningococcic serum with the meningococcus at the temperature of from 37° to 38° C. The substitution of a water-bath of that temperature for the thermostat at 55°C., as employed in the usual agglutination tests with meningococci, is a great simplification, especially under conditions of Army work.

5. The simultaneous yielding of pure cultures of meningococcus for stock or further study, provided all the steps have been conducted in a strictly sterile manner.



## FLUID SUBSTITUTES FOR TRANSFUSION AFTER HEMORRHAGE.

### FIRST COMMUNICATION.

BY PEYTON ROUS, M.D., AND GEORGE W. WILSON, M.D.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

There exists at present a great and urgent need for an injection fluid that can be satisfactorily employed instead of blood for transfusion in cases of hemorrhage. It is common knowledge that casualty clearing stations, after a "push," are crowded with men who have lost too much blood to be operated on, who cannot be revived by means of salt solution and supportive measures, but who would undoubtedly respond to transfusion. For the latter neither time nor donors are available.

A number of fluid substitutes for transfusion have recently been suggested. Our work relates, first, to what may be expected of a substitute, and, second, to the relative merits of some of the substitutes proposed.

### *The Limits of Substitution for Hemoglobin.*

That severe acute hemorrhage, even to apparent exsanguination, does not entail permanent damage to the organism is shown by the rapid and complete recovery that usually follows transfusion. May one expect some such recovery when another fluid than blood is used? Or is this essentially dependent on the new supply of corpuscles and plasma?

The importance of the plasma has been investigated by Abel, Rowntree and Turner,<sup>1</sup> who demonstrated that the healthy body will withstand and quickly repair great losses of the fluid. The ability to withstand similar losses of red cells only—or, more properly speaking, of functioning hemoglobin—has not been inquired into. This

1. Abel, J. J., Rowntree, L. G., and Turner, B. B.: Jour. Pharmacol. and Exper. Therap., 1914, 5, 625.



point was the first taken up in our work. Animals have been bled repeatedly, and the blood bulk at once restored by the injection of compatible plasma.

Rabbits were used because their rapid exsanguination and injection involves only minor operative procedures. All possible blood was taken by cardiac aspiration with strong suction, and then through an ear vein warmed plasma was run in containing the minimum of sodium citrate that would prevent clotting (about 0.5 per cent.). At the first bleeding, somewhat more than half of the total calculated blood bulk (5.5 per cent. in the rabbit<sup>2</sup>) was usually obtained in from one to three minutes. It was replaced with exactly the same amount of plasma. After a wait of a few minutes, to give time for part of the citrate to leave the circulation, a second bleeding and injection was carried out. Thus from three quarters to four fifths of the total hemoglobin was abruptly replaced.

The results were surprising. When the hemoglobin was reduced by not more than three fourths—as, for example, from 80 to 20 per cent.—and the rabbit replaced in its cage, no marked change was noted in its behavior. The respirations were for a few minutes somewhat quickened, and the ears for many hours were cold, with vessels contracted; yet the animal moved about normally, and immediately after the operation would eat when supplied with food. The blood gradually returned to normal in the course of the next few weeks, and the only inconvenience noted was that during the days of severest anemia, the least exertion would cause the animal to pant heavily.

A very different condition was seen after four fifths or slightly more of the hemoglobin had been removed. On its release from the operating board, the animal lay panting, with the accessory muscles of respiration actively employed, and made at first no attempt to rise. Soon, however, the respirations became less frequent, though still rapid, and when touched the animal would get on its feet. At first, following the outside stimulus, it sat like a normal rabbit with head up, ears back, and legs drawn well together; but soon the head nodded, the ears drooped, the legs sprawled out irregularly, and if undisturbed the animal sat somnolent for a long time. When touched

2. Boycott, A. E., and Douglas, C. G.: *Jour. Path. and Bacteriol.*, 1909, 13, 256.

it would straighten up and assume an alert posture, only in the course of a few minutes to relax again. These animals died in from three to six hours.

We have spoken of proportional amounts of hemoglobin rather than of absolute quantities, because of indications that, within limits, it is the proportional loss that most affects the results. A rabbit with an initial hemoglobin of 80 per cent., Sahli, will withstand a reduction to 20 per cent., whereas this is fatal to an animal having 100 per cent. to begin with. The symptoms described can scarcely be due to the citrate in the plasma, since this was varied freely in cases yielding similar results, and because, furthermore, the same effects were obtained when a noncitrate fluid, namely, horse serum, was used instead of plasma. The lowest absolute hemoglobin percentage to which we have reduced an animal without death was from 17 to 18 per cent., Sahli. Horse serum was the fluid used to replace the blood withdrawn. In this instance, the hemoglobin value did not rise during several days.

The ability of rabbits in which anemia is gradually produced, as well as that of patients with chronic anemia, to survive with only 20 per cent. of hemoglobin or less is usually ascribed to gradual bodily adjustments. But the present experiments show that in reality the body can tolerate an abrupt change to a very low figure.

Such great losses of hemoglobin as were accomplished in our rabbits are never brought about by hemorrhage. As is well known, a loss of slightly more than half the total blood bulk is usually fatal to man. This fact is true also of rabbits. The loss of hemoglobin is never so great as that of blood bulk, for the reason that as hemorrhage proceeds the blood is diluted with fluid previously extravascular. The dilution occurs so rapidly that the last blood to flow from a cut carotid is appreciably thinner than the first. Thus a relative retention of hemoglobin is brought about. This point is well indicated in the recent work of Depage and Govaerts.<sup>3</sup> They studied the hematologic indications for transfusion on wounded soldiers, and state that when wounds do not involve the abdomen, and the red cells fall below 4,500,000 per cubic millimeter in the first three hours, or

3. Depage and Govaerts: *Presse méd.*, 1917, 25, 602.

4,000,000 in eight hours, or 3,500,000 in the first twelve hours, the patient will probably die unless transfusion is performed. In such instances, the full loss of hemoglobin is doubtless not indicated by the counts, since there is an attendant loss in blood bulk. Yet, even allowing for this, the total reduction in hemoglobin is not sufficient to account for the impending death. The amount of the pigment retained in the body under the circumstances of the most severe acute hemorrhage is always far above that which, as the present experiments have shown, will support life. The objection may be made that under the circumstances of clinical hemorrhage, no such immediate replacement of blood bulk occurs as in our bled and infused rabbits, and that the losses of hemoglobin may not be so well supported by animals rendered acutely anemic for some hours. But, as has already been pointed out, the results of transfusion in exsanguinated individuals show clearly that ordinary hemorrhage does not of itself produce lasting injury.

The view, therefore, seems justified that, however desirable it may be, it is not essential to supply blood corpuscles in ordinary cases of *acute* hemorrhage. Even in the worst example, the body retains at least twice the minimum functioning hemoglobin which, if other factors are favorable, will support life.

### *The Limits of Substitution for Plasma.*

To determine the limits of possible substitution for plasma is difficult for the reason that the body possesses immediate fluid reserves as well as the ability to manufacture fresh plasma in a few hours. This, of course, is not the case with hemoglobin. Only after the depletion of the fluid reserves does an injected liquid play an essential part in an animal's recovery. And even then the results must be discounted because of the organism's activity in furnishing for itself a new plasma. The limits of substitution are directly dependent on the time taken for the plasma's removal and that allowed for recovery.<sup>4</sup> When both are generous, the possibilities as regards plasma

4. Hurwitz, S. H.: Intravenous Injections of Colloidal Solutions of Acacia in Hemorrhage, *THE JOURNAL A. M. A.*, March 3, 1917, p. 699. Abel, Rowntree and Turner (Footnote 1).

withdrawal and substitution are practically unlimited. Unfortunately, in cases of hemorrhage the depletion is extremely rapid and involves both cells and plasma. New fluid is required, not merely to replace the plasma, but also to make up the total blood bulk.

### *Blood Bulk and Blood Substitutes.*

Hemorrhage depletes, not merely the blood, but the fluid reserves as well—a fact long recognized by physiologists. The results may be clearly seen in rabbits bled at intervals of fifteen minutes. The effect on a good sized animal of a first bleeding of 20 c.c. is to lower the blood pressure only temporarily. A second similar bleeding causes a greater fall and a somewhat slower recovery, but soon the normal is reached again. A third bleeding of only 10 c.c. may now bring about a great drop in pressure, from which there is practically no return. In such an instance, not only have all physical means of adjustment, such as vasoconstriction, been pressed into play, but the fluid reserves have been so exhausted that nothing is forthcoming to replace the blood bulk. Under such conditions, the effects of a substitute for the blood can be directly observed.

Unfortunately, the fluid reserves and other compensatory mechanism differ so much in animals of the same size that often one cannot tell how far recovery is dependent on the character of an injected fluid and how far on the organism's natural resiliency. For this reason, as well as to save time, we have employed the rapid exsanguination by suction already referred to. Half or more of the total blood—and blood not diluted as when it flows from a vessel—is rapidly removed and replaced through an ear vein with the fluid under test. Aspiration from the femoral artery has proved safer than that from the heart, and quite as rapid. A large blunt needle is thrust far up the vessel, and then suction applied. The blood pressure is recorded from the carotid artery with mercury manometer and kymograph.

The ability of plasma to replace whole blood was first studied. When more than half of the total calculated blood volume had been taken, and the carotid pressure had fallen to a physiologic zero (from 10 to 20 mm. of mercury), it was instantly and permanently

restored to the normal by the injection of an equivalent quantity of plasma. This was the case, too, when horse serum was used. A saline solution, on the other hand (Ringer's fluid), brought about only a slight, transient recovery of the pressure. Sometimes, nevertheless, the animal survived for the half hour or more necessary for the successful utilization of its own fluid resources.

The reasons for the failure of saline solution, as thus indicated, have been variously given, but the most important, undoubtedly, is that it leaves the vessels for the tissues and the urine almost as soon as it is injected. The same is true to a less extent of many fluids better than salt solution, but not so good as plasma. After the injection of such a fluid, the blood pressure may be immediately restored to normal; but it does not remain there. Almost at once the pressure begins to fall as the fluid leaves the vessels, and only after a greater or less period, as the organism itself furnishes a blood substitute, does the fall cease and the pressure rise gradually to normal. If the fluid is a fairly good substitute for blood, the depth of the curve expressing the pressure changes is not great (from 15 to 20 mm. of mercury), and return to the normal pressure is complete within one-half hour.

### *The Relative Merits of Blood Substitutes.*

Gelatin-containing fluids have often been used as blood substitutes. Hogan<sup>5</sup> advises 2.5 per cent. gelatin and Bayliss,<sup>6</sup> 6 per cent. We have tested several fluids made with gelatins which answered to Hogan's requirements for purity and ability to "gel." They were made up carefully according to his formula, and rabbits were used as in his experiments. But though these solutions undoubtedly restored blood pressure better than did salt solution, their effect was soon lost, the pressure falling off in the course of fifteen or twenty minutes nearly to the level to which the bleeding had originally reduced it. In these results there is no necessary contradiction to Hogan's findings. He took the rate of secretion of urine as the

5. Hogan, J. J.: The Intravenous Use of Colloidal (Gelatin) Solutions in Shock, THE JOURNAL A. M. A., Feb. 17, 1915, p. 721.

6. Bayliss, W. M.: Proc. Roy. Soc., B, 89, 380.

index of the ability of his solution to stay in the vessels, and found that it was not increased. But, as Bogert, Underhill and Mendel<sup>7</sup> have shown, loss of fluid into the tissues is often far more important for blood volume than that through the kidneys.

Larger concentrations of gelatin up to 6 per cent. act proportionately better than Hogan's solution. In some instances, 4 per cent. gelatin restores blood pressure permanently, but in others it does not. Six per cent. gelatin, in our experience, is always effective.

It has been interesting to determine the effects of hypertonic glucose and dextrin solutions, since these have been advised to draw fluid into the circulation. Under most circumstances, a pump for continuous injection, such as that employed by Erlanger and Woodyatt,<sup>8</sup> will not be available, so we have contented ourselves with immediate large injections. Eight per cent. dextrin (Merck) in salt solution, and 5.4 per cent. glucose in Ringer's solution—a fluid with twice the tonicity of blood—have been found to exert only a slight transient effect to raise the blood pressure in the bled rabbits.

Bayliss, who originally advised either 6 per cent. gelatin or 7 per cent. gum acacia as blood substitutes, has recently advocated the latter,<sup>9</sup> and for good reasons. It can be sterilized by boiling, whereas the autoclaving of gelatin is necessary in order to kill tetanus spores. It is more uniform in constitution than commercial gelatin, and, being protein free, it does not induce anaphylaxis or the severe reactions that often follow the latter. We can confirm Bayliss' statement that 7 per cent. gum acacia will permanently restore blood pressure to normal.

At the British front, where acacia solutions are now used, there has been an avoidance of the higher percentages in favor of a 2 per cent. fluid, and the results, while encouraging, have not been convincing. The reason for this is well seen in our experiments. A 2 per cent. acacia solution, like Hogan's fluid, at first raises the pressure to normal, but it drops off within a few minutes to the danger point.

7. Bogert, L. J., Underhill, F. P., and Mendel, L. B.: *Am. Jour. Physiol.*, 1916, **41**, 219.

8. Erlanger, Joseph, and Woodyatt, R. T.: *Intravenous Glucose Injections in Shock*, *THE JOURNAL A. M. A.*, Oct. 27, 1917, p. 1410.

9. Bavliiss, W. H.: *Arch. méd. belge*, 1917, **70**, 793.

Four per cent. is more satisfactory, as the secondary drop in pressure, being slow, is better compensated. But neither the 4 per cent. nor the 5 per cent. solution recommended by Hurwitz<sup>4</sup> is effective in all cases. Six or 7 per cent. is required if one is to bring back the normal pressure in an organism depleted of its fluid reserves.

Mention has already been made of horse serum. The indications are that it would be an effective blood substitute, except for the risk of inducing sensitization or causing anaphylactic shock. In nonsensitive animals (rabbits, dogs), it acts as well as the plasma of the same species. But large quantities of it bring about serum sickness. A rabbit that had three quarters of its blood replaced by horse serum died on the sixth day, and showed extensive liver necroses. In a sensitive man, as we have had opportunity to observe, the injection of 2 c.c. induced a fall of twenty points in the systolic pressure, which was repaired only after several hours. In a nonsensitive man, the effect of the injection of 90 c.c. was to raise the pressure slightly for a few minutes. Horse serum injections, under the conditions of the battlefield, might well save many lives, but they would probably cause death in some cases.

### *Blood Substitutes and the Individual Case.*

The needs for a blood substitute may in individual cases be widely different. When the hemorrhage has been rapid and has been completely checked, almost any harmless isotonic solution will tide the patient over. It matters little that the fluid will soon leave the vessels, for the patient's own fluid reserves are almost intact, as is his ability to manufacture a plasma rapidly. At the other extreme are those instances in which the blood has been draining steadily away and there remains in the body no source of an immediate restoration of fluid. Here half-measures cannot suffice. A fluid must be furnished which will take the place, over many hours, of the lost blood bulk. Except for the blood or plasma of other human beings, fluids containing from 6 to 7 per cent. of gum acacia are the best at present available for the purpose. Their use when death threatens and transfusion cannot be performed is worth the taking of risks. Intermediate cases can undoubtedly be much helped by a 2 or 3 per cent.

acacia solution or perhaps by Hogan's solution. In view of our ignorance of the after-effects of these foreign substances, it is certainly advisable not to inject more than the needs of the case may demand.

Further attempts to devise blood substitutes may be expected in the next months. The fact may, therefore, be pointed out that it is not vital to success, as Bayliss has assumed, that a fluid substitute have the viscosity of whole blood; for plasma, which has only about half this viscosity, substitutes perfectly. Indeed, the advisability of raising the blood pressure after hemorrhage beyond a certain point by making an injected fluid difficult to push through the vessels may well be questioned. In order that a diminished number of red cells may carry on the body's normal work, it would seem that a brisker circulation is requisite.

Our experiments have brought out no indication of a special stimulating influence on blood pressure of either plasma or serum. That these fluids possess special characters which render them preferable to any possible substitute is obvious. But the important point is that their use is not essential.

#### SUMMARY.

The animal organism will withstand an abrupt reduction in hemoglobin to almost, if not quite, the low percentage that is tolerated in chronic anemia. Roughly speaking, three quarters of the total hemoglobin may be safely removed, provided the blood bulk is maintained. If four fifths is suddenly withdrawn, the animal becomes apathetic, shows symptoms of air hunger, and dies in a few hours. The amount of hemoglobin which remains after fatal acute hemorrhages is far above the minimum requirement of the body. As Abel and his co-workers have shown, great losses of plasma are soon repaired by the organism, if only the blood bulk is maintained. Taken together, these facts show that however desirable transfusion may be (especially to furnish the elements needed in clotting, to lessen acidosis, to improve oxygenation, etc.), it is not essential to recovery from even the severest *acute* hemorrhage, if only the blood bulk can be restored in other ways.



Of the several fluid substitutes for transfusion which have recently been suggested, all are preferable to salt solution. Bayliss' 7 per cent. gum acacia solution is up to the present the best, and its use should save life in many instances. In less urgent cases, from 2 to 3 per cent. acacia solution, or Hogan's 2.5 per cent. gelatin solution, are to be preferred to salt solution. But these fluids leave the circulation relatively soon, and when the organism has been drained of its fluid resources their injection restores the blood pressure to the normal level for only a few minutes. Permanent betterment cannot be expected in cases of severe hemorrhage with solutions containing less than from 5 to 7 per cent. gum acacia.

It is not essential that a blood substitute should possess the viscosity of whole blood.

## THERAPEUTIC EXPERIMENTS WITH ROSENOW'S ANTI-POLIOMYELITIC SERUM.

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PLATES 8 TO 10.

(Received for publication, January 2, 1918.)

Investigators are divided into two well defined groups with reference to the interpretation of the relation of certain streptococci cultivated from poliomyelitic nervous tissues to epidemic poliomyelitis. One group<sup>1</sup> affirms that the streptococci bear a causal relation to poliomyelitis and are even related biologically to the globoid bodies of Flexner and Noguchi,<sup>2</sup> while another group<sup>3</sup> denies that they possess any essential etiologic importance and views them merely as secondary invaders.<sup>4</sup>

The question at issue is an important one in every way, because upon its true answer will depend the prophylactic measures adopted to prevent epidemics of poliomyelitis and the direction which effort will take in perfecting an efficient agent for specific therapy.

Until recently the effort made to treat cases of poliomyelitis specifically has been with the blood serum of convalescent and recovered cases of the disease. This procedure is based upon several kinds of conclusive experimental data. However, too few observations are available to decide whether the method gives unmistakable thera-

<sup>1</sup> Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202; *Science*, 1916, xlv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

<sup>2</sup> Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

<sup>3</sup> Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557. Kolmer, J. A., Brown, C. P., and Freese, A. M., *J. Exp. Med.*, 1917, xxv, 789.

<sup>4</sup> Smillie, W. G., *J. Exp. Med.*, 1918, xxvii (in press).

peutic results in human cases of poliomyelitis, although the indications are favorable.<sup>5</sup>

The employment of the serum derived from recovered cases of poliomyelitis followed not only on account of the detection of its neutralizing property *in vitro* for the poliomyelitic virus, but also because of the failure to induce antibody formation in a variety of domestic animals, including the horse, by the injection of the nervous tissues of monkey or man carrying the virus.<sup>6</sup> Incidentally, it may be stated that only imperfect success in developing antibodies in rabbits and monkeys has attended the repeated injection of cultures of the globoid bodies.<sup>7</sup>

A far greater measure of success has been claimed for the streptococci in producing antibodies for the virus of poliomyelitis. Rosenow<sup>8</sup> and Nuzum and Willy<sup>9</sup> assert that animals immunized with the streptococci cultivated from poliomyelitic cases exhibit various antagonisms to the virus. Monkeys inoculated with streptococci are said to be protected from subsequent infection with the poliomyelitic virus; the blood of the protected monkeys is stated to be neutralizing *in vitro* for the virus; and finally, horses immunized with the virus are said to yield a serum which possesses neutralizing, protective, and therapeutic properties, even when applied to man.

It is this last statement which calls for painstaking control. It is obvious that to obtain a decision from the treatment of human cases of poliomyelitis would require a large and varied series of observations extending over a long period of time and embracing epidemics of considerable magnitude and various degrees of severity. Moreover, the observations would have to be carefully controlled by comparison with an equal number of cases, occurring simultaneously, untreated with the antiserum, under approximately identical conditions and equally accurately studied. To secure these data might require several years, as has been the case notably with the serum treatment of diphtheria and epidemic meningitis. The question arises,

<sup>5</sup> Amoss, H. L., and Chesney, A. M., *J. Exp. Med.*, 1917, xxv, 581.

<sup>6</sup> Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

<sup>7</sup> Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 545.

<sup>8</sup> Rosenow, E. C., *J. Am. Med. Assn.*, 1917, lxix, 261, 1074.

<sup>9</sup> Nuzum, J. W., and Willy, R. G., *J. Am. Med. Assn.*, 1917, lxix, 1247.

therefore, whether a method is not available by means of which a probable decision may be reached more expeditiously and with greater certainty. It is because of our belief that a decisive experimental method is at hand that the series of experiments to be reported were performed.

#### EXPERIMENTAL.

The injection intracerebrally into monkeys of minute quantities of an active virus of poliomyelitis is followed by paralysis and, as a rule, by death of the animal. The injection of far greater quantities of the same virus into the blood stream produces no symptoms. If, however, as Flexner and Amoss<sup>10,11</sup> have shown, the meninges and choroid plexus are chemically inflamed by a simultaneous or previous injection of sterile horse serum, monkey serum, or even isotonic saline solution, the virus is enabled to pass from the blood into the nervous tissues and thus to induce the characteristic changes which lead to paralysis and even to death. The same authors found only one substance which, when injected intraspinally, prevented the localization of the virus in the nervous organs after intravenous injection, and that is the serum derived from monkeys which have survived a poliomyelitic infection. Moreover, this serum is capable of setting aside the effects of the chemical inflammation incited by horse serum or other foreign fluids injected intraspinally. In other words, when the immune convalescent serum is introduced into the meninges in animals previously injected intraspinally with horse serum or other fluids mentioned, followed by an intravenous injection of the virus, no paralysis or other evidence of infection results. This experiment gives such decisive and unequivocal results that it seems particularly adapted to determine the therapeutic value of a serum or other product reputed to be effective in the treatment of poliomyelitis in man.

The experiments to be described were carried out in the following manner. An active, fresh poliomyelitic virus was obtained in the usual manner by inoculating a monkey intracerebrally with a suspension of glycerolated virus. On the 1st day of complete prostration, the animal was etherized, and the brain and spinal cord were

<sup>10</sup> Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, **xx**, 249.

<sup>11</sup> Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, **xxv**, 525.

aseptically removed. With the spinal cord and medulla, a 5 per cent suspension in isotonic salt solution was prepared, shaken, and centrifuged, and the clear supernatant fluid injected intravenously into *Macacus rhesus* monkeys. Two separate sets of experiments were performed. Control animals and animals treated with Rosenow's serum, with normal horse serum, and with convalescent monkey serum, in the same manner, were tested simultaneously. The protocols follow. The outcome is as sharp and hence as decisive as the experimental results could make it.

*Experiment 1.*—Monkey A, control. Nov. 13, 1917. Injected intravenously 50 cc. of the virus prepared as described. The animal remained well.

Monkey B, normal horse serum control. Nov. 12, 1917, 5.10 p.m. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 13, 11.15 a.m. Injected intravenously 50 cc. of virus. 12 noon. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 14. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 15. Repeated intraspinal injection of normal horse serum. Nov. 16. Repeated intraspinal injection of normal horse serum. Protects left leg; ataxic. Nov. 17, a.m. Left arm, right deltoid, and both legs paralyzed; head tremor; ptosis of the left eyelid; almost prostrate. 4.30 p.m. Died.

*Autopsy.*—Macroscopic and microscopic lesions of poliomyelitis. No visible changes in viscera.

Monkey C, Rosenow's serum. Nov. 12, 1917, 4.40 p.m. Intraspinally injection of 3 cc. of Rosenow's antipoliomyelitic horse serum activated with one-ninth volume of fresh guinea pig serum. Nov. 13, 10.45 a.m. Intravenous injection of 50 cc. of virus. 10.50 a.m. Intraspinally injection of 3 cc. of Rosenow's serum. Nov. 14, 15, 18, 19, and 20. Injected 3 cc. of Rosenow's serum, intraspinally. The clinical course of the animal was as follows: Nov. 17. Slow. Nov. 19. Excited; slow; protects right leg. Nov. 20. Drags right leg; climbs awkwardly. Nov. 22. Both legs weak. Nov. 23. Legs paralyzed; deltoids weak. Nov. 24. Prostrate (Fig. 1). Nov. 26. Moribund; etherized.

*Autopsy.*—The spinal cord and brain were edematous and the gray matter was congested. Microscopic examination of the central nervous system showed marked perivascular infiltration and some neurophagocytosis in the gray matter of the medulla and cervical enlargement characteristic of poliomyelitis. Perivascular infiltration (Figs. 2 and 3), congestion, neurophagocytosis, and meningeal infiltration in lumbar enlargement. Focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in the posterior root ganglia.

Monkey D, serum of recovered monkeys. Nov. 12, 1917, 4.55 p.m. Intraspinally injection of 3 cc. of mixed serums from several *rhesus* monkeys which had recovered from experimental poliomyelitis and subsequently received subcutaneous injections of the virus contained in the spinal cord and medulla (reinforced

immune). Nov. 13, 11 a.m. Intravenous injection of 50 cc. of virus suspension followed by intraspinal injection of 3 cc. of immune serum. Nov. 14, 15, 18, 19, and 20. Intraspinal injections of 3 cc. of immune serum. The clinical course of this animal was in striking contrast with the preceding. At no time were any symptoms present; the animal continued apparently normal throughout the treatment and is well at the present time (Jan. 1, 1918).

This experiment was repeated with precisely the same results. The protocols follow. In the second experiment, the normal horse serum control was omitted.

*Experiment 2.*—Monkey E, control. Nov. 26, 1917. Intravenous injection of 50 cc. of the virus. No symptoms appeared, and the animal has remained normal up to the present time (Jan. 1, 1918).

Monkey F, Rosenow's serum. Nov. 26, 1917, 6 p.m. Intraspinal injection of 2 cc. of activated Rosenow's antipoliomyelitic horse serum. Nov. 27, 11.35 a.m. Intravenous injection of 50 cc. of virus, followed immediately by the intraspinal injection of 2.5 cc. of Rosenow's serum. The intraspinal injections were repeated on Nov. 28, 29, Dec. 2, and 3. In each instance the activated serum was injected. Dec. 3. The animal developed a marked tremor of the head, ataxia, and right facial paralysis; also, the deltoid muscles were weak. Dec. 4. The monkey died in the early morning.

*Autopsy.*—Macroscopic lesions of poliomyelitis throughout brain and cord. Microscopic examination of the central nervous system showed marked congestion and perivascular infiltration, slight cell degeneration, and neurophagocytosis in medulla and cervical enlargement (Fig. 4); slight meningeal infiltration in lumbar enlargement, and focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in posterior root ganglia (Fig. 5).

Monkey G, immune monkey serum. Nov. 26, 1917, 5.15 p.m. 2 cc. of pooled immune serum injected intraspinally. Nov. 27, 12.25 p.m. Intravenous injection of 50 cc. of the virus, followed by the intraspinal injection of 2.5 cc. of pooled immune serum. The intraspinal injections of the pooled serum were repeated on Nov. 28, 29, Dec. 2, and 3. At no time were any symptoms detected, and the animal is normal at this time (Jan. 1, 1918).

#### DISCUSSION.

The preceding experiments accomplish two purposes directly. First, they test the ability of Rosenow's serum, which was prepared by injecting a horse with cultures of the streptococci derived from poliomyelitic nervous organs, to prevent a poliomyelitic infection arising in the monkey after an intravenous injection of the virus. This is readily accomplished by means of the immune serum obtained

from convalescent and recovered monkeys. Second, they compare directly under these favorable therapeutic conditions the Rosenow serum with the serum of immune monkeys.

The results of the experiments are unequivocal. They show the Rosenow serum to be devoid of protective power. Moreover, they show that the Rosenow serum acts in the manner of normal horse serum in promoting infection in monkeys from an intravenous injection of the virus, in itself incapable of inducing paralysis.

The immune monkey serum possesses, under the same conditions of administration, perfect protective power, as has been shown previously by Flexner and Amoss.<sup>11</sup>

A further conclusion may be drawn from the experiments. Rosenow states that the horse serum prepared by him contains demonstrable antibodies for the streptococci employed in its production. It is assumed that these antibodies are identical with the antibodies, demonstrable by neutralization experiments with the virus, contained within human and monkey serum derived from recovered cases of poliomyelitis in man and the monkey. This supposition is rendered untenable by the results of our experiments. The antibodies induced in the horse by immunization with the streptococci have proven incapable of neutralizing the virus of poliomyelitis introduced into the blood of monkeys in its passage to the central nervous system—a neutralization which the immune monkey serum readily effects. The two classes of antibodies or immunity principles, those present in the blood derived from recovered cases of poliomyelitis and those induced in the horse by treatment with streptococci, are therefore to be regarded as distinct.

There is a further corollary to this general deduction. Once it is established that the antibodies yielded by the streptococci differ essentially from those induced by the virus of poliomyelitis, the contention that virus and streptococci are identical becomes untenable. In other words, the experiments reported in this paper tend also to refute the claim that certain streptococci are the microbic cause of epidemic poliomyelitis.

Rosenow and Nuzum and Willy assert that their serums possess striking therapeutic activity in man. Their conclusions are based on the treatment of relatively small numbers of cases of epidemic polio-

myelitis during the past summer and autumn. We have already drawn attention to the difficulties surrounding a statistical study, of limited extent, of the questions here involved. Hence we venture to place, in this instance, the greater weight on decisive animal experiments, and those reported in this paper clearly show that Rosenow's horse serum injected intraspinally into monkeys is without specific protective power against the virus of poliomyelitis.

It has, however, been shown by Flexner and Amoss<sup>12</sup> that normal horse serum, when injected intraspinally into monkeys, promotes the passage of poliomyelitic immune bodies from the blood into the subarachnoid space. Hence it is possible that under certain circumstances in which those bodies are already present in the blood in man, they may be directed into the subarachnoid space through the increased permeability of the meninges induced by the horse serum and thus affect the course of the infection. The antibodies have been detected on the 3rd<sup>13</sup> and 6th<sup>12</sup> days of illness in man, or, in other words, early in the course of the disease.

This, however, is a purely hypothetical consideration, in support of which normal horse serum should prove as effective as antistreptococcus serum. It is questionable whether this roundabout method of directing the circulating immunity bodies to the central nervous organs is advisable in practice. As far as present knowledge, based on definitive experiments, is concerned, it may be said that only immune serum derived from convalescent and recovered cases of poliomyelitis in man and the monkey have been determined to be protective against the infectious power of the poliomyelitic virus.

#### CONCLUSIONS.

Two series of experiments are described in which Rosenow's anti-poliomyelitic serum, so called, has been compared with the immune serum derived from monkeys which have convalesced or recovered from experimental poliomyelitis.

The experiments consisted in introducing an active virus of polio-

<sup>12</sup> Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

<sup>13</sup> Kling, C. A., and Levaditi, C., *Études sur la poliomyélite aiguë épidémique* Paris, 1913, p. 114.



myelitis into the blood and of injecting the two kinds of serum into the cerebrospinal meninges according to the method of Flexner and Amoss.

Under the conditions of the experiment, the control monkeys (*a*) receiving the virus intravenously alone do not develop paralysis, while those (*b*) receiving the virus intravenously and normal horse serum intraspinally develop paralysis. Moreover, the monkeys (*c*) receiving the virus intravenously and Rosenow's antipoliomyelitic serum intraspinally develop paralysis in the manner of those receiving normal horse serum intraspinally. The monkeys (*d*) which received the virus intravenously and the convalescent or immune monkey serum intraspinally alone did not develop paralysis.

The Rosenow serum acts in the manner of normal horse serum; it promotes the passage of the virus of poliomyelitis from the blood into the nervous organs, and it does not protect from infection.

We have found no evidence that Rosenow's serum under the conditions of the tests is effective therapeutically in monkeys or possesses antibodies of the same nature as those present in the blood of monkeys which have recovered from experimental poliomyelitis.

Since the antibodies in convalescent poliomyelitic serum in man and the monkey are identical, it follows that any antibodies present in the Rosenow horse serum do not conform to those occurring in human convalescent serum.

#### EXPLANATION OF PLATES.

The illustrations were taken from monkeys treated with Rosenow's serum.

##### PLATE 8.

FIG. 1. Monkey C. 11 days after the intravenous injection of virus. Received seven intraspinal injections of Rosenow's serum. Arms, legs, and back muscles paralyzed; face muscles active.

##### PLATE 9.

FIG. 2. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in anterior horn.  $\times 165$ .

FIG. 3. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in posterior horn.  $\times 165$ .

PLATE 10.

FIG. 4. Monkey F. Cervical enlargement showing anterior horn with degeneration of ganglion cells and neurophagocytosis.  $\times 240$ .

FIG. 5. Monkey F. Posterior root ganglion with ganglion cell degeneration, neurophagocytosis, and mononuclear cell infiltration.  $\times 240$ .



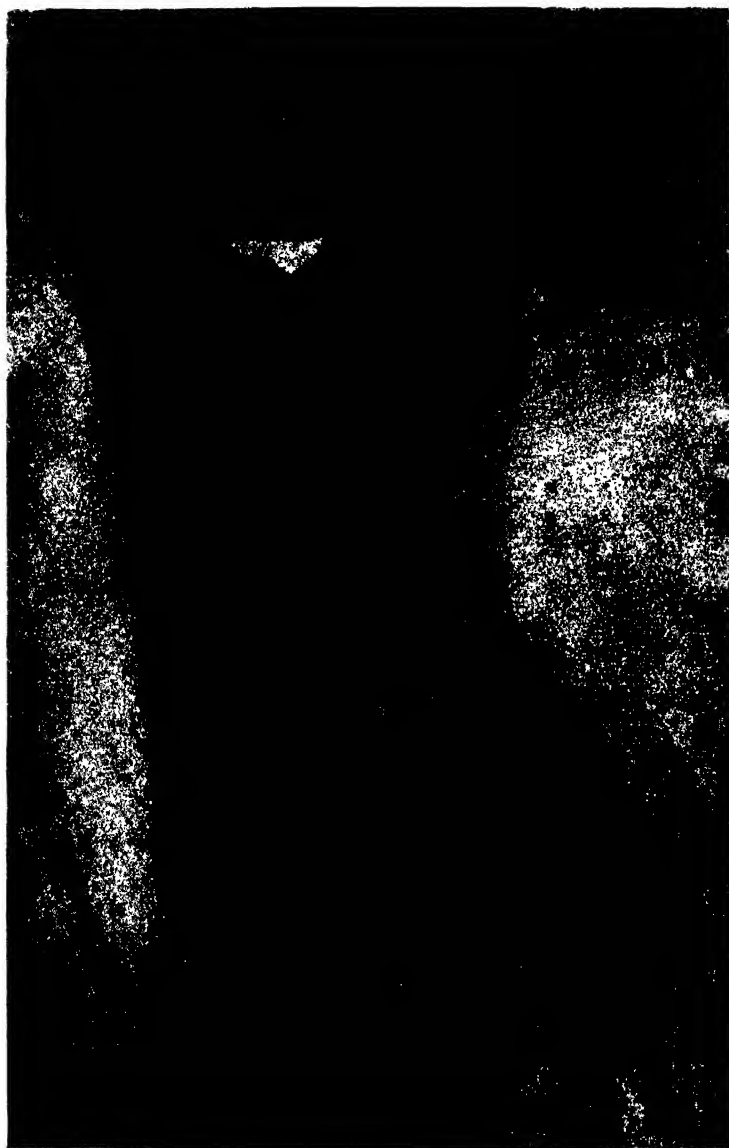


FIG. 1.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)



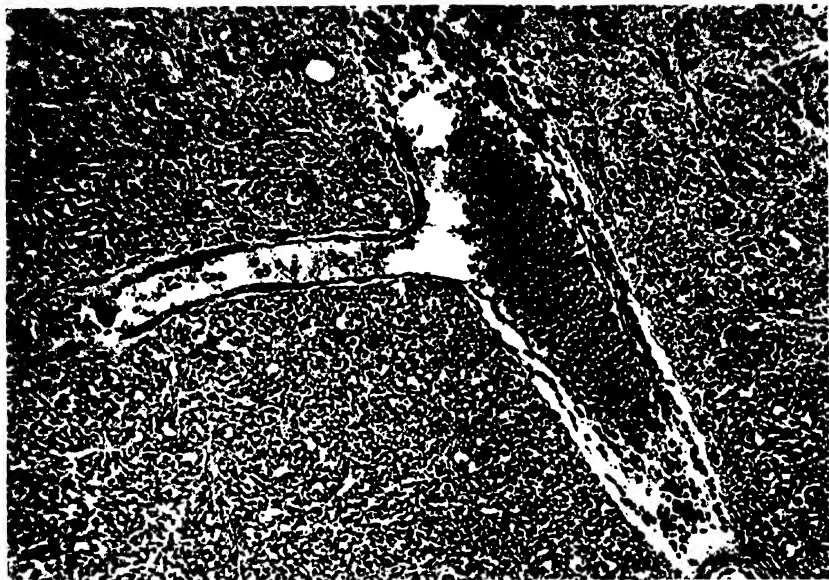


FIG. 2.



FIG. 3.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum..



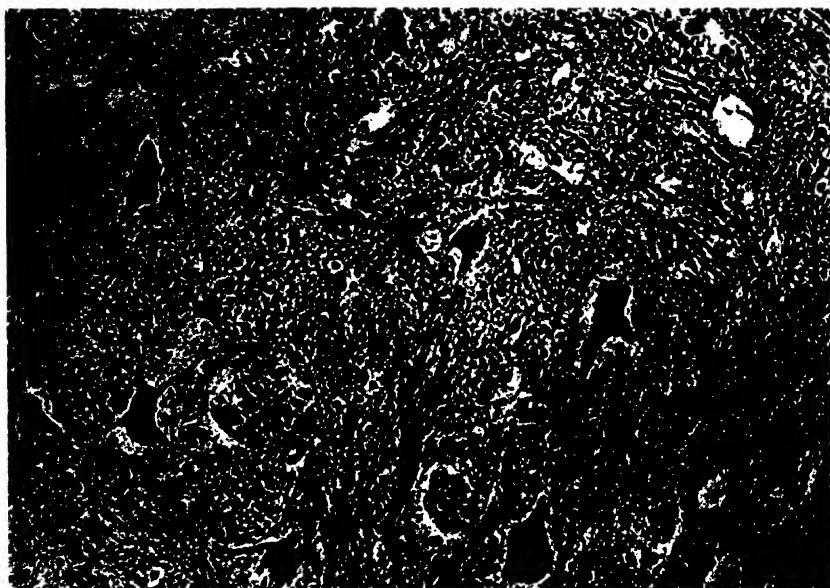


FIG. 4.

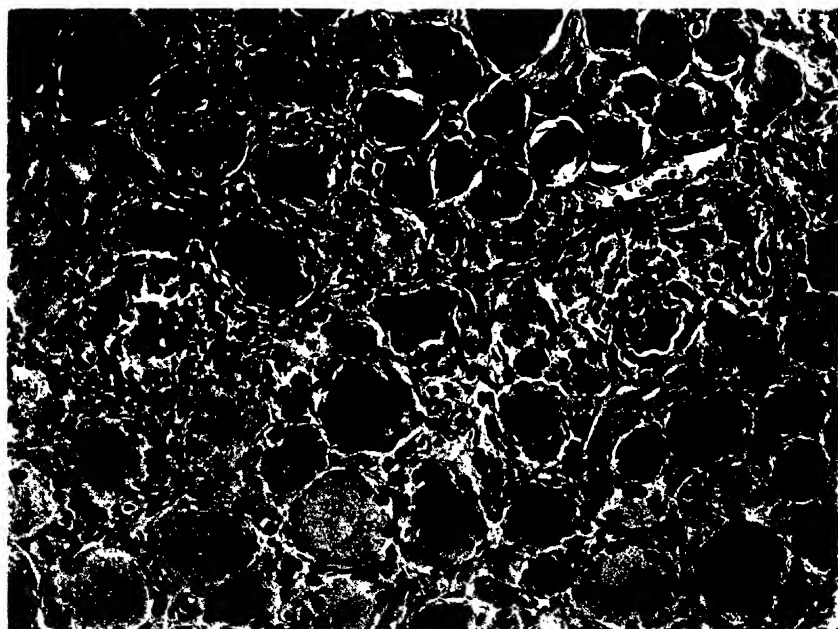


FIG. 5.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)





## IS THE INFLUENCE OF THYMUS FEEDING UPON DEVELOPMENT, METAMORPHOSIS AND GROWTH DUE TO A SPECIFIC ACTION OF THAT GLAND?

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The experiments on thymus feeding thus far reported in the literature have given results sufficiently different to prevent the formation of a definite idea as to the rôle of the organ in these experiments. This is even true if one has in mind only one of the various groups of animals which have been studied in such experiments. Concerning the Amphibia among which only the larvae of Anura have been studied carefully regarding their reaction to thymus feeding, it seems that most of the experiments showed a retarding influence of the thymus upon development and metamorphosis although some exceptions are reported. With respect to growth however, the results are so lacking in uniformity, that Gudernatsch as well as Romeis who studied the effect of thymus feeding in tadpoles doubted whether the effect produced by this organ was due to a specific action or only to quantitative conditions. Gudernatsch in his experiments on tadpoles noted accelerated growth leading to enormous size; but Romeis obtained completely normal growth in various series of thymus-fed tadpoles and pointed out that the thymus feeding never produces abnormally large animals.

The following experiments which will be reported elsewhere in detail, seem to indicate that the accelerated growth of thymus-fed Amphibian larvae is merely the effect of quantitative conditions and not the result of the specific quality of the organ such as a specific growth-stimulating agent. Furthermore, they yielded some very interesting results concerning development and metamorphosis although these are still difficult to explain. Finally they showed that in each thymus-fed larva severe tetany is produced. The last mentioned phenomenon will be discussed in another article; the effects of thymus upon development, metamorphosis and growth will be outlined briefly in the following pages.

In the experiments to be reported, only larvae of Urodela were studied (*Amblystoma punctatum* and *A. opacum*). The advantage of using Salamander larvae is that the quantity of food given to them can be controlled and measured with exactness, up to a certain degree, which cannot be done if tadpoles are used; and it should be emphasized that in order to avoid errors the possibility of measuring the quantity of food is very desirable and even should be demanded in experiments in which it is suspected that qualitative relations are involved.

### *1. Development and Metamorphosis.*

Gudernatsch found that the development of tadpoles was delayed if the animals were fed on thymus. Similar results were obtained by Romeis in his first experiments. This led to the inference that thymus contains a substance whose specific property is a retarding effect upon development. Only recently Gudernatsch again published a paper based on this hypothesis.

However, in his work published in 1915 relative to the influence of the glands of internal secretion on *Anura* larvae, Romeis reports upon two series of experiments, which cannot be explained from the above-mentioned standpoint, and which caused the author himself to doubt whether inhibition of development were indeed a specific function of the thymus. The first series consisted of fairly old larvae of the species *Rana esculenta*, the individuals of which developed in a perfectly normal manner, in spite of being fed with thymus; but in this case it might have been supposed that the thymus feeding had been started too late. In the second series, however, in which larvae of *Rana temporaria* were employed, the thymus feeding was commenced at a very early stage, in spite of which fact the development of the larvae was not delayed. On the contrary, they underwent metamorphosis at an earlier stage than did the larvae fed with muscle tissue, and before the latter had developed front limbs. This last series of experiments suggests the assumption that the influence exerted by the thymus on development must be dependent on factors not specific for the thymus. The experiments on Salamander larvae now to be described led to similar conclusions.

Both Gudernatsch and Romeis took as index of the rate of development, the growth of the hind and front limbs, the absorption of the tail and the abandonment of the water. The latter phenomenon however, which we will refer to as metamorphosis seems in Salamanders, to be dependent on a mechanism different in many respects from that which controls development, such as the growth of limbs, etc.; for in the first place even under conditions of normal feeding, different individuals show a different stage of development when they leave the water, and secondly the effect of thymus upon development and upon metamorphosis does not seem to be the same in the Salamander larvae examined. Therefore we shall distinguish between development and metamorphosis; growth and differentiation of the limbs, certain changes of the fin and gills not being in direct relation to the abandonment of the water, and the changes of the color pattern of the skin which finally lead to the definite coloration of the skin, will be referred to as development; while the abandonment of the water together with the sudden reduction of the gills to mere stumps and the complete absorption of the fin will be called metamorphosis.

In a group of eight series, O. 1916, in which larvae of *Amblystoma opacum* were used, four series were fed with small fragments of earthworms and four series with equal sized pieces of thymus. As will be explained later on, these experiments were conducted with the intention of feeding the respective animals with equal quantities of worms and thymus. So far as development and metamorphosis is concerned, it would seem at least possible, that besides the quality of food, the amount of food may also have some influence upon these two phenomena; but at any rate only if we make the quantities of food alike in the experimental series and the controls, can we be sure that the differences obtained in both series are the expression of the quality of the food.

The development of the legs and toes was carefully noted, and the development of these organs was seen to occupy a period of from 6 to 9 weeks. As the thymus feeding began as early as the second week, the development of the legs took place under the influence of thymus feeding during 4 to 7 weeks. According to Gudernatsch and Romeis this length of time sufficed in the case of tadpoles to

produce the retarding effect upon development of the thymus; but in the case of the Salamander larvae absolutely no retardation could be noted as a consequence of the thymus feeding. Indeed, in those series which as a result of the simultaneous effect of a lowered temperature necessitated a longer period of time in order to attain complete development, a change in the contrary direction could even be noted in the latest stages, the thymus animals attaining complete development of their limbs more quickly than the worm-fed animals.

Thus it can hardly be argued in this connection that feeding had not lasted long enough to produce a result, in view of the fact that in the latest stages of development of the legs when the feeding had lasted a longer period than in the first stage, the opposite result was obtained. This result then indicates that a distinct difference exists between the Anura and Urodela with respect to the effect of thymus-feeding upon development.

The development of the legs in such animals to which instead of equal quantities of food as much food was given as each animal was able to take, was not studied in sufficient detail. In one group of experiments (P. 1917) which consisted of *A. punctatum* larvae, development of the legs was recorded during 14 days after the beginning of the feeding; in this group the relation between the thymus-fed animals and the controls was the same as in the above experiments on *A. opacum*.

The differences between the Anura and Urodela become even more accentuated as development proceeds. But a careful distinction must be made between animals fed on equal quantities and those which obtain as much food as they will take.

In the above mentioned group (O. 1916) which consisted of larvae of *Amblystoma opacum* fed with equal quantities, the development of further advanced characteristics of the legs, of the shape of the head, of the gills and of the color of the skin proceeded much more rapidly in the thymus-fed animals than in those fed on worms.

With respect to the development of the gills, the following should be remarked: In larvae of *Amblystoma opacum* fed with worms and kept at an average temperature of 22.6°C. as late as one or more days before metamorphosis the gills attain a stage not only of con-

siderable size, but one in which they are characterized by considerable redness and above all by the fact that they are bent upwards in a crescent shape. The long well developed branches are widely extended and the points of the stem inclined forwards so as to bend over. In worm-fed animals kept at a high temperature (22.6°C.) this condition of the gills was only attained in the 23rd week, but in thymus-fed animals kept at the same temperature as early as the 11th week; in worm-fed animals kept at a low temperature (in average 14.8°C.) only in the 29th week; in thymus-fed larvae kept at the same temperature as early as the 11th week (although in the latter case the gills were less developed than in the case of the high temperature thymus-fed animals).

A similar relationship is observed with respect to the color of the skin. In the case of the warm worm-fed animals the melanophore spots only began to develop in the 13th week, at which time they had already attained very considerable development in the case of the warm thymus-fed animals; in the warm worm-fed individuals the blue-grey pigment did not appear until the 24th week, but in that of the warm thymus-fed animals as early as the 12th week. In the cold worm-fed animals the fusion of the melanophore spots into a uniform black-brown coat only began in the 30th week, and occurred as early as the 13th week in the case of the cold thymus animals; but in the cold worm-fed animals no trace of a silver-grey pigment can be detected after 32 weeks, although this appeared in the cold thymus-fed individuals as early as the 13th week. These differences in the rate of development are doubtless sufficiently great to indicate distinctly the differences existing between the Anura and Urodela. Under conditions of quantitatively equal feeding (which alone can be taken into consideration in a study of qualitative effects) the feeding of thymus to larvae of *Amblystoma opacum* causes accelerated development.

Nevertheless, the above mentioned experiments become even more clear if the results obtained by them are compared with the results in experiments made by a different method; for the factor to be emphasized is not the time elapsed since the hatching but the size of the animals. The thymus-fed individuals attain the stated conditions of development while much smaller in size than the worm-

fed animals. The latter must attain much greater size than the thymus-fed animals, in order to acquire the same degree of development.

In a group of *A. punctatum* (P. 1916) kept at a high temperature, one series was fed with pieces of thymus and another with Tubifex. In both series the animals were allowed to eat according to their inclination as a result of which the worm-fed animals consumed a considerably larger quantity of food than did the thymus-fed animals, and consequently grew much more rapidly. The development of the skin pigmentation also proceeded more quickly than in the case of the thymus animals, although the latter attained these various stages while much smaller in size. At the time no exact drawings were made to show the relationship between the size and stage of development. This will be taken up in a recently initiated experimental series of *A. punctatum* (P. 1917) as yet incomplete, in which the same system of feeding is being maintained as in Group P. 1916. Meanwhile it can already be noted that the worm-fed animals do not develop the yellow network until they have attained the average size of 62.91 mm., the minimum length being 59 mm. The thymus animals, which have only attained an average length of 32.22 mm., with a maximum length of 36 mm., have not yet shown signs of this network. In group P. 1916 of *A. punctatum* in which the worm-fed animals behaved like the worm-fed animals in Group P. 1917 regarding the relation between size and development of network, the first thymus animal attained the network stage when only 41.3 mm. in length.

We thus see that the time at which the various phases of development are attained varies according to the quantity of food, with the result that sometimes the thymus animals, at other times the worm-fed animals appear to lead. But the constant factor is the size at which the various stages are attained; that is, constant to the extent that the thymus animals always develop more quickly than do the worm animals, if the various stages are referred to the size of the animals. This relationship is directly opposed to that of the *Anura* larvae, for in these animals the thymus-fed individuals must usually attain a considerably greater size than the worm-fed animals in order to arrive at the same degree of development.

Identical relationships as occur in the development are also found in the metamorphosis; but in this case one or more additional factors seem to play a rôle to complicate considerably the phenomena, as we shall see.

Here again we must differentiate between the experiments in which the food was quantitatively equal and those in which each animal was allowed to eat to the point of satiety. But it should be emphasized that only the first method permits of a correct comparison. For when the worm animals feed at will they eat approximately 10 to 20 times the quantity of food that is consumed by the thymus animals when the latter begin to suffer from tetany; as the worm animals also grow much more rapidly as a result, it would not be surprising that they also metamorphose earlier, since we might expect that if a definite size of the animal is indispensable to metamorphosis, metamorphosis will be accelerated if we accelerate growth by some external conditions.

We will now turn our attention again to the group O. 1916 of *A. opacum* in which each series was given approximately the same quantity of food. In this group the warm thymus animals were the first to undergo metamorphosis; thus in the warm thymus series (22.6°C.) the first animal underwent metamorphosis in the 13th week, in the warm worm series only in the 24th week; in the cold thymus series (14.8°C.) the first animal left the water in the 24th week; whereas in the cold worm series no animal has yet undergone metamorphosis (in the 32nd week). Thus, thymus-fed animals are seen to metamorphose earlier than worm-fed animals; that is, provided they receive equal quantities of food.

The relationship of time however becomes inverted if the worm and thymus animals, instead of receiving equal quantities of food are allowed to eat at will. In a group of *A. punctatum* (P. 1916) consisting of two series, which had been kept at a high temperature and in which the last-mentioned mode of feeding was adopted, the first animal of the thymus series underwent metamorphosis after 5 months, whereas the first of the worm series did so after only  $3\frac{1}{2}$  months.

As in development, so also in metamorphosis, the relationship of time is seen to be inconstant and depends on the amount of food



given to the animals. But a constant factor exists in the relationship between size of the animal and metamorphosis. Whatever method of feeding may be adopted, the thymus-fed individuals are always much smaller when they undergo metamorphosis than are the worm-fed ones. In the Opacum group (O. 1916) consisting of equally fed animals, the warm thymus animals averaged only 47.8 mm. in length at the time that the first individual underwent metamorphosis, whereas in the worm series at the beginning of metamorphosis the average size was 53.5 mm. The same relationship can be observed at a low temperature; the average size of the thymus animals being only 57.5 mm. at the beginning of metamorphosis, whereas the worm animals had not yet begun to metamorphose when their average length was 65.1 mm. The same conditions apply in the above-mentioned Punctatum series (P. 1916); the thymus animals begin to metamorphose when their average size is 41.9 mm., but the worm animals only at an average size of 50.0 mm.

As in the case of development, so in metamorphosis the relationships obtaining in *A. opacum* and *punctatum* are exactly the reverse of those found in the *Anura* larvae, for in the former the worm-fed animals must attain a much greater size than the thymus-fed individuals before they can undergo metamorphosis, whereas in the case of the *Anura* larvae the thymus animals must be larger than the worm animals before metamorphosis can occur.

However, in addition to the facts mentioned above, still another phenomenon must be described which seems to aid greatly our understanding of the relation between development and metamorphosis. If we refer metamorphosis neither to the time which has passed since hatching nor to the size of the animals but to the stage of development of certain structures, metamorphosis does not appear to be accelerated in the thymus animals but rather retarded.

For example, when comparing the warm worm animals with the warm thymus animals of the Opacum group (O. 1916) we see that as early as the 11th week the warm thymus animals attained the same stage of development at which the warm worm animals commenced to metamorphose. At this stage, however, a remarkable phenomenon is noted; the warm thymus animals fail to metamorphose while some of their organs continue to develop; the structures

of their skin, which are responsible for the development of the color of the skin, attain while the animal is still larval a phase of development reached by the worm animals only some time after metamorphosis has been accomplished. After the warm thymus animals have entered upon the stage characterized by the crescent-shaped gills and the fusion of the melanophore spots, they should, if compared with controls, undergo metamorphosis, but instead they develop the silver-grey pigment and undergo reduction of the size of the fin. Simultaneously (a point to be specially emphasized) they stop growing and become reduced in length, a condition which also occurs in the case of worm animals before metamorphosis. They assume an aspect which on the whole resembles that of a worm-fed animal which had undergone metamorphosis about two weeks previously. As can already be seen, these relationships can be noted much more distinctly in the cold Opacum series; but as the animals of these series have not yet all undergone metamorphosis and the worm animals have not yet begun to metamorphose, we will not describe the phenomenon already noted. Exactly the same phenomenon can be seen in a group of Punctatum (Group P. 1916) maintained at a high temperature, such as the development of definite characteristics of a metamorphosed animal during the larval stage. In this case the yellow network was separated into yellow spots during the larval stage—a phenomenon which does not occur in the case of the worm animals before they have left the water.

From what has been stated above we can see that even in those animals which metamorphosed first and, in the series of Opacum larvae (O. 1916) fed with equal quantities, metamorphosed 11 weeks earlier than the worm animals, the process of metamorphosis was disturbed. This becomes much more apparent if for the date at which the first animal underwent metamorphosis we substitute that of the last animal metamorphosed. In that case we obtain the following relationship: In the series of Opacum larvae (O. 1916) after 32 weeks have passed, 12 per cent of the thymus-fed animals are yet in a larval stage, whereas the worm-fed individuals all had metamorphosed as early as the 29th week. Thus, the period of metamorphosis in the worm series extended only over 5 weeks, whereas in the case of the thymus animals it has already lasted

19 weeks. In the repeatedly mentioned group of *A. punctatum* larvae (P. 1916) kept at a high temperature, the last thymus-fed animal had not left the larval stage even after 8 months, whereas the last worm-fed animal had metamorphosed after only  $5\frac{3}{4}$  months; thus in the worm-fed animals, metamorphosis covered a period of only  $2\frac{1}{3}$  months, whereas in the thymus animals it lasted 5 months. In a group of *A. punctatum* (P. 1916 C) kept at a low temperature, a worm-fed series comprising individuals which out of a number of 300 larvae had not yet undergone metamorphosis was added to a thymus-fed series which had been under observation for about 5 months. In other words, this worm-fed series consisted of larvae which were abnormally late in undergoing metamorphosis. The first of these worm-fed animals left the water  $5\frac{3}{4}$  months after hatching, the last  $7\frac{2}{3}$  months after hatching, the period of metamorphosis extending in this series over 2 months. Of the thymus-fed animals the first metamorphosed after  $4\frac{1}{3}$  months the last (leaving two animals out of consideration) after  $6\frac{1}{3}$  months. In the case of these thymus-fed animals the period of metamorphosis lasted 2 months, for instance, not longer than in the case of the worm animals; but 2 of these thymus animals not yet mentioned, behaved very differently from all the other animals. They both remained at a low stage of development, so far as coloring was concerned, and their tails underwent but slight reduction in size. On the other hand, the gills were reduced to short stumps. Although neither of these 2 animals was shedding its skin (which should take place before metamorphosis) at the time of the reduction of the gills, they were taken out of the water and placed in a vessel, the bottom of which was covered with filter paper and just enough water to keep the vessel wet. But neither of the animals showed any further change, until finally  $12\frac{2}{3}$  months after hatching one of them shed its skin and its gills became completely atrophied, while at the same time the skin became darker in color although the yellow network failed to develop. The other animal is still in the larval stage,  $13\frac{2}{3}$  months after hatching.

We must not fail however to mention that it still appears very doubtful whether this is a direct effect of thymus, for a similar phenomenon was also noted in the case of worm-fed animals, although not to so extreme a degree. Out of approximately 300 worm-fed

animals, only 1 individual showed such a condition; after more than 8 months it was still in a larval condition and had not developed a trace of the yellow network. The fin of its tail was but slightly reduced; its gills were more reduced and the animal was still undergoing growth and taking food spontaneously. It was used for the purpose of an operation, in the course of which it died. However, as has been said, at this stage it showed no trace of approaching metamorphosis. From this it seems very doubtful that the delay of metamorphosis in the two last mentioned thymus animals was actually due to the action of thymus and we must exclude them from discussion until the same phenomenon is obtained in a greater number of cases.

## 2. *Growth.*

In one series of experiments (P. 1917) for which purpose larvae of *A. punctatum* which had hatched on the same day and were the offspring of the same mother were employed, it was assumed that where there is an unlimited supply of food, the amount spontaneously taken up by each animal is a function of growth, and that growth is not a function of the food quantity. For that reason in these experiments which were carried out at an average temperature of about 22°C., the animals were allowed as much food as they felt inclined to take.

The group consisted of three series. The animals of the first series were given small equal-sized fragments of thymus with a pair of forceps, until each animal was satisfied. They took the pieces easily and owing to the softness of the material had no difficulty in swallowing them. The second series received fragments of earth-worms. Owing to the hardness of this food, however, the animals found great difficulty in swallowing it, and it took several minutes, or even hours for each piece to be swallowed. As they were fed only once a day, these worm animals remained hungry and consequently were soon backward in growth, as compared with the thymus-fed animals. The latter finding coincided with the observations made in the case of the *Anura*; i.e., that the thymus stimulates growth; but it failed to prove a specific influence of thymus, for the reason that the animals which were fed in a normal manner were found

to be starving. In a third series the animals were fed with small worms (*Enchytraeus*), which were at first given in small pieces; these worms were thrown into the containers in such large quantities that the animals never lacked food. Besides this, each animal was fed on pieces of earth-worms which the fast-growing animals soon took readily and in large quantities. The individuals of this series grew faster from the very outset than did the thymus animals. As the latter did not develop tetany until the 5th week and were in a completely normal condition until the end of the 4th week, we may look upon the result attained up to that time as the pure effect of nutrition. The Salamander larvae failed to show that the thymus had exerted any growth-accelerating influence. On the other hand, the quantity of food given plays an important part in this connection, for the animals react in a highly sensitive manner to relatively slight differences in food quantities. From these experiments it would seem that in the experiments on tadpoles conducted by Gudernatsch and Romeis the factor revealed is not a specifically growth-promoting influence, but that the accelerated growth of the thymus animals should be attributed to the fact that the jaws of the tadpoles, although adequate to supply the body with a quantity of the soft thymus material corresponding to the needs of the organism, were nevertheless not the most appropriate instrument for preparing from the hard beef muscle sufficient nutriment for the purpose of keeping up normal growth. The very fluctuating results which Romeis obtained in his experiments indicate pronounced sensitiveness on the part of the tadpoles to small quantitative differences of food which often completely escape control, rather than the presence in the thymus of a specific growth-promoting influence. It should also be remarked that in the above-mentioned experimental group it was also noted that the animals fed with worms must consume a much greater quantity of earth-worms than the thymus-fed animals consume of thymus in order to grow equally quickly; the supply of earth-worm fragments which the second series consumed was only slightly smaller than that of the first series fed with fragments of thymus. This fact speaks in favor of relatively high nutritive value in the thymus. It should also be taken into consideration that in the fragments of earth-worms a not inconsiderable part

of the volume consumed consists of indigestible substances (chitin, earth) which are later eliminated in the feces.

In the preceding order of experimentation it is seen that at the moment that the tetany period begins in the thymus-fed animals we are confronted by an obstacle which prevents any quantitative judgment from being formed; for from this time on the thymus animals are seen to be abnormally placed and the amount of food taken in by them becomes abnormally low. This is all the more disturbing for the reason that it is uncertain whether under these conditions the quantity of food spontaneously taken is really a function of growth. On the contrary it appears very probable that the reduced amount of food taken must be attributed to disturbances caused in the swallowing apparatus by the convulsions. In such a case the animals would be in a condition of starvation and in contradiction to the idea of the experiment the rate of growth would be the function of the food quantities introduced into the organism. I hope to be able to overcome this obstacle in another group of experiments, in which I proceeded from the fact that when food is present in sufficient quantities equal amounts of food produce an equal rate of growth.

In a group consisting of 4 series (O. 1916) for which larvae of *A. opacum* were used, the food was given in small fragments at the point of the forceps in all the series; an attempt was made to make all the pieces of approximately the same size on the same day of feeding. The number of pieces given to each individual animal was noted, and on each feeding day approximately (for the week) the same number of pieces was given, so that all the animals of these 4 series received approximately the same number of pieces, the series comprising one thymus and one worm group at an average temperature of 22.6°C., and one thymus and one worm group at about 14.8°C. An effort was hereby made to distribute a quantitatively equal amount of food among the 4 series; but it must be remarked that this can only be roughly attempted and cannot be exactly carried out. As it can never be known beforehand how much food the animals may need on a given day in order to be satisfied, it would also be quite impossible to weigh the food. But even if this were possible, the distribution of equal quantities according to weight would not

lead to the distribution of equal nutritive quantities as a given volume of thymus contains a larger quantity of substances available for metabolism than does the same quantity of fragments of earthworms, as has been shown in the first experimental group. Although this method is not exact, it has at least furnished an approximate idea as to how important it is to control the quantity of food in such experiments.

Of course the quantity of food to be given each day was always standardized from the series which desired the smallest amount to eat. At the beginning these were the worm series, and of these the cold worm series showed less avidity for food than did the warm worm series. As a result the thymus series at first received less than they would have liked to eat. The reasons for this comparatively small appetite in the worm animals have been specified above when discussing the first experimental group. From the time that the series of warm thymus animals began to undergo metamorphosis, the animals of this series showed the least desire to eat; after that it was the worm animals in general, and the warm worm series in particular which received less than they could have consumed.

It may be emphasized at this point that when this method of distributing quantitatively equal amounts of food is followed, tetany exerts a very slight influence on growth. Sometimes the rate of growth is reduced at such points where the tetany curve reaches its apex, but in other cases, on the contrary it increases or reaches even a maximum when the tetany curve does.

The condition which exerts an influence on growth in comparison with which all other influences are reduced to insignificance, is metamorphosis, as will be apparent from the following description.

During the first few weeks the warm thymus animals are seen to lead in size; next in order come the cold thymus animals, then the warm worm series, and finally the cold worm series. Nevertheless no special importance must be attributed to this relationship, for as has already been stated, given an equal volume of food, the thymus animals probably obtain more nourishment from their pieces of thymus than do the worm animals from an equal quantity of worm fragments. The relationship of size which has just been mentioned lasts until the 10th week, and the acute tetany which has meanwhile

set in among the warm thymus animals and reached its climax has failed to influence this relation at all. In the 11th week a pronounced change sets in; at this stage the warm thymus animals are all ready for metamorphosis, the first individuals being 14 days removed from this step. During this week the curve of the body size of the cold thymus animals, which up to that time occupied the second position, can be seen to cross that of the warm thymus animals. From the time that the first animal of the warm thymus series entered upon metamorphosis, the warm thymus animals completely stopped growing. Their curve, which of course does not include the metamorphosed animals, is soon after crossed by those of the two series of worm animals, and the warm thymus animals remain smallest in size for the rest of the experiment.

The cold thymus series, the first individuals of which underwent metamorphosis in the 24th week, also increase in size only a little from the time of metamorphosis on; but as the first animals of the warm worm series which is most proximate to the cold thymus curve similarly undergo metamorphosis in the 24th week, and also because the curve of the cold thymus animals is higher above that of the warm worm animals than the curve of the warm thymus animals is above the cold thymus animals, the curve of the latter remains the first at the beginning; it is not crossed by that of the warm worm animals until the latter have all metamorphosed. Finally, in the 29th week, together with the curve of the warm worm-fed animals, it is crossed by the curve of the cold worm series, which now occupies the first position. As early as the end of the 29th week the largest animals of the cold worm series have attained a size greater than that of each non-metamorphosed (and of course of each metamorphosed) individual of the three remaining series. As for the present (after the 32nd week) the animals give no sign of impending metamorphosis and continue to grow.

The above-reported circumstance appears to us to be the most instructive with reference to the statement that thymus-fed anuran larvae attain a size by many denoted as abnormally large but stated by Romeis never to exceed normal limits, although sometimes exceeding the size of the muscle-fed animals. If we begin by comparing each of the two thymus series with the corresponding worm



series, we see that the same relation exists between them as between muscle- and thymus-fed tadpoles, inasmuch as the animals which metamorphose later attain greater dimensions than do those which first underwent metamorphosis. It can be seen that this is not connected with a specific influence of thymus feeding, from the fact that exactly the same relation exists between the warm and the cold worm series, the warm worm-fed series which first underwent metamorphosis metamorphosing while smaller in size than the cold worm series, and the latter continuing to grow after the former has metamorphosed. While yet in the larval stage the cold worm-fed animals attain a size which when the largest animals of both series are used for comparison, already exceeds that of the largest warm worm-fed larva by 12.5 mm.<sup>1</sup> From another point of view the Salamander larvae of those species so far examined show the very opposite characteristics from those possessed by the anuran larvae; for it is not the worm-fed salamander larvae which first undergo metamorphosis but the thymus-fed individuals. Thus, the point to be primarily emphasized is not the greater size ultimately attained by the worm-fed Salamanders and thymus-fed tadpoles, for we have seen that this does not depend upon the specific qualities of the thymus, but that it is a general phenomenon peculiar to amphibia and one dependent upon the time at which the animals undergo metamorphosis. The point of importance in both cases—the larvae of Anura as well as of *A. opacum* and *A. punctatum* is the circumstance that thymus-feeding produces metamorphosis in the Anura only when considerable size has been attained, whereas in the Urodela, on the other hand, this occurs while the animal is but small in size.

To summarize, we may make the following statement: The differences in the rate of growth to be noted before metamorphosis are not the result of a specific growth-promoting influence of the thymus; they are based on the circumstance that animals which are

<sup>1</sup> Although the cold worm larvae are at the time of writing larger than the largest metamorphosed warm worm animals, we do not here intend to take up the question of this relation; moreover, a comparison of the experiments hitherto conducted in connection with the Anura shows this not to be possible, as the respective authors never observed their experimental animals beyond the period of metamorphosis.

better fed grow more quickly. In the experimental group of *A. punctatum* (P. 1917) discussed in the preceding section, these individuals are obviously the worm-fed animals of the third series, which are allowed to have as much food as they wish; in the experimental group with *A. opacum* (O. 1916) it is the thymus animals which take in a greater quantity of nutritive material through eating thymus. The experiments furthermore show that qualitative influences exerted on the rate of growth would have to be very considerable in order that they can be experimentally tested in the case of amphibia, for in these animals the slightest quantitative differences, such as can hardly be controlled, would bring about very misleading differences in growth.

With respect to the ultimate size attained by the animals, Salamander larvae resemble tadpoles in the fact that under certain conditions the later they metamorphose the greater is their final size; this is not only true for thymus-fed animals in comparison to worm-fed animals, but also for worm-fed animals kept in high temperature in comparison to worm-fed animals kept in low temperature.

The action of thymus on development and metamorphosis may be summarized in the following way:

In animals fed on thymus the development presumably of the organism as a whole but certainly of the legs, gills, shape of the head and color of the skin, is greatly accelerated during the larval period. The thymus-fed animals, therefore, reach the stage at which worm-fed animals are ready for metamorphosis, much more quickly than worm-fed animals. As development at least to some degree may be dependent on growth, on the rate of growth and on size, it is impossible to examine the specific influence upon development of any substance without keeping alike the conditions of growth in both the experimental and control series; such was attempted by admitting an equal amount of food to both series.

When the thymus animals have reached the stage at which worm-fed animals go into metamorphosis, the development of most organs seems to stop, while certain characteristics of the skin continue to develop; the skin of such animals then behaves very similarly to the sex-organs of neotenic larvae, since the skin at least with regard to the structures determining pigmentation, develops charac-

teristics of a metamorphosed animal, while the animal as a whole still is in a larval stage. At the time when metamorphosis should occur disturbances in the course of development begin to appear evidently due to the suppression of the development of some factor, without which further development is impossible. In most of the animals of a thymus-fed series this factor still develops much earlier than in the controls; but even in these individuals metamorphosis becomes a grave danger to the animal's life. In high temperature some animals die during metamorphosis and those which survive metamorphosis die a relatively short time after metamorphosis. In some individuals the development of the factor necessary for metamorphosis is still more disturbed and becomes delayed in comparison with the controls; at high temperature all individuals in which this is the case die on the day when the gills and the rest of the fin undergo the sudden reduction in size, characteristic of the entrance into metamorphosis. In low temperature they may survive metamorphosis. In low temperature a very small percentage of the thymus-fed animals may remain at a low stage of development and not metamorphose for more than a year; but whether this is due to the action of the thymus diet is not yet certain, as a similar phenomenon was observed in one worm-fed animal of the stock.

It seems that we cannot understand the results reported in thymus feeding experiments if we assume that they are the pure expression of the influence of the thymus substance. The rather great fluctuations reported in individuals of the same species as well as the surprising differences between larvae of *Anura* and *Urodela* when fed on thymus, indicate that quite a number of factors are involved in metamorphosis, some of which were not controlled in the experiments. It is of course clear, that differentiation of the organism is one of these factors; that a certain degree of differentiation is indispensable for metamorphosis, or at least to facilitate it, was shown by Gudernatsch in some recent experiments on the influence of thyroid. That some of the individuals among a thymus-fed series of Salamander larvae metamorphose earlier than the controls may be due in some degree to the fact that in the thymus-fed Salamander larvae development and differentiation and consequently metamorphosis also depend on the general conditions of growth; the ex-

periments on Salamander larvae reported suggest that rate of growth and size play an important rôle in metamorphosis. The difference noted between Anura and Urodela when fed on thymus can be explained only by assuming a fundamental difference between the organization of these two groups of animals. It will be pointed out in another article that such a difference, namely the absence in the Salamander larvae and the presence in the anuran larvae of the parathyroids, seems to explain why thymus-feeding should develop tetany in Salamander larvae and should not in anuran larvae. It suggests itself that metamorphosis in part must depend on a factor similarly being present in one group but absent in the other group. The development of that factor may be induced primarily by processes occurring in a certain stage of differentiation, but also may be influenced and inhibited or disturbed by thymus diet. The action upon this factor of the thymus may be widely different from that upon developmental processes preceding its development; this is indicated by the fact that development while accelerated during the larval period is on the contrary retarded from the time at which metamorphosis should occur. It is this phenomenon which emphasizes the fact that metamorphosis to some degree must occupy a particular place among the processes of development. In this connection, finally, frequent reports may be remembered according to which thymus causes disturbances of the blood circulation; in metamorphosis of the Amphibians the blood circulation undergoes a fundamental change in the course of which the gills are absorbed, and in Salamanders, the absorption of the gills according to Maurer, is a prerequisite for the formation of the parathyroids. It may be worth while to keep these facts in mind during further studies of the influence exerted upon metamorphosis by the thymus.

Though the effect of thymus feeding on development and metamorphosis is very evident, it appears to the writer that similar effects may be produced by other and purely quantitative external conditions, such as temperature and quantity of food and in general by all factors which modify growth, rate of growth, size and velocity of development. No doubt such factors are of great importance in determining at what time, at what size and developmental stage

of the animal, metamorphosis will occur. Since the relations between these different factors are very complicated and the number of experiments relative to them is rather small, discussion of these conditions must be postponed.

Finally it should be mentioned that the thymus gland apparently contains all substances which are necessary to build up the substance of an Amphibian organism to maintain the animal growing and to sustain life permanently. This is demonstrated by a number of specimens of *A. punctatum* kept at low temperature which have been fed on thymus since about the 14th day of their life and are now about 14 months old; they are increasing in size.

## CULTIVATION EXPERIMENTS ON THE GLOBOID BODIES OF POLIOMYELITIS.\*

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The microbic cause of poliomyelitis is a subject still in the foreground of interest with respect to the etiology of that disease. The present paper deals with the conditions surrounding the cultivation of the globoid bodies described by Flexner and Noguchi (1) from poliomyelitic tissues and is intended as a contribution to the technique of the method.

The technique of the cultivation is a complex and difficult procedure, even more difficult, perhaps, than the isolation of *Treponema pallidum*. With respect to the treponema, the experimenter is assisted by the fact that it is comparatively large, possesses clear-cut morphological characteristics, and is motile—properties which make its detection relatively easy.

The globoid bodies, on the other hand, are extremely minute and non-motile. They are not readily distinguished from tiny particles of detritus in the early generations of scantily growing cultures. Hence weeks, even a month or more, may elapse between the initial inoculations of the culture medium and the definite determination of successful cultivation of the organism; and it may happen that the growth then observed may fail to develop on transplantation, so that the opportunity for its identification may be lost. The difficulties surrounding the method of cultivation have resulted in the confirmation of Flexner and Noguchi's work by only a few bacteriologists, the larger part of those who have attempted to repeat it failing in their efforts.

The present study was undertaken in the hope of modifying the

\* This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

method by rendering it simpler and more certain of success. The original procedure is not only uncertain in its ultimate results, but also in the number of cultures yielded by a given lot of tubes inoculated with poliomyelitic tissue. Sometimes of 30 to 50 tubes thus inoculated, one or two only would yield cultures, the others remaining without demonstrable growth. Where the conditions all appear to be so similar, it would seem that a higher percentage of cultures should be obtained. Obviously the conditions within the tubes varied in an unaccountable manner; and it became also the purpose of this study to eliminate the factor of variation and thus to increase the proportion of cultures obtainable from a certain number of inoculated tubes.

Hitherto, the globoid bodies have been cultivated from the central nervous organs, which are the seat of the profounder effects of the disease. Now, according to present conceptions of the pathology of poliomyelitis, the disease partakes often of the nature of a general or systemic infection. Indeed, the non-paralytic or abortive cases, so called, pursue a course unattended by paralysis or even by marked symptomatic involvement of the central nervous system. Hence it became desirable to study the general viscera by culture methods.

The source of the tissues submitted to cultivation was *rhesus* or *cynomolgus* monkeys in which the inoculation form of the disease had been produced. The usual procedure was to permit the paralysis to develop to the point at which the inoculated animals became prostrate; they were then etherized. In certain instances animals were used which had been prostrate for several days or which had died some hours earlier. A comparison of the cultures from the two classes of animals—those recently paralyzed and still in good physical condition with those which have been *in extremis* for some time or have actually succumbed to the disease a number of hours before autopsy—is illuminating as regards the number and kind of cultures obtained.

The virus or emulsion of the nervous organs employed for inoculation was either an active virus which has been transmitted in the laboratories of The Rockefeller Institute for several years, or the less active M.A. virus (2), which has also passed through many monkeys. The mode of inoculation was usually intracerebral, but

sometimes it was by other routes—the nose and blood. A brief summary of the clinical data of the infected monkeys is given in Table I.

We propose to give the details of the technique of the successful cultivations of the globoid bodies, which will be followed by a description of the results obtained by the method, including both success and failure, and the probable causes of each.

TABLE I.  
*Summary of Clinical Data of Monkeys Infected with Poliomyelitic Virus.*

Serial No.	Source of virus.	Amount inoculated.	Route of inoculation.	Duration of symptoms.	Duration of prostration.	Length of illness.	Pathologic appearance.
				days	days	days	
1	Fresh mixed.	50.0 cc.	Intravenous.*	1½	½	7	Typical.
2	Glycerolated mixed.	2.0 cc suspension	Intracerebral.	4	1	13	"
3	Fresh mixed.		Nasal.	9	6	18	"
4	" "	0.2 cc. filtrate.	Intracerebral.	3	1	10	"
5	" "	50.0 cc.	Intravenous.*	2	0	7	"
6	" "	0.1 "	Intracerebral.	1½	½	10	"
7	" "	0.1 "	"	1	0	11	"
8	" "		Nasal.	3	1	11	"
9	" "	0.1 cc.	Intracerebral.	2	1	7	"
10	" "	0.2 "	"	8	1	13	"†
11	" "	0.2 "	"	7	3	15	"†
12	M.A.	1.0 "	"	5	1½	9	"
13	Fresh mixed.		Nasal.	4	2	12	"
14	" "		"	2	½	10	"
15	" "	2.0 cc.	Intracerebral.	2	1	6	"
16	" "		"	2	½	6	"
17	" "		Nasal.	3	1	7	"
18	M.A. cultivated.	2.0 cc. mass culture.	Intracerebral.	5	0	12	"

\* Intravenous inoculation of centrifuged emulsion following the injection of horse serum intraspinally.

† These animals had been dead 7 and 3 hours respectively when autopsied.

### *Cultivation Technique.*

The methods used for the cultivation of the globoid bodies from the tissues of monkeys are essentially the same as those originally



described by Flexner and Noguchi (1). Certain variations of the technique will, however, be described. In part, the technique is a repetition of previously reported work, but is given as a convenient summary.

Test-tubes, 1.5 by 20 cm., should be used for all experiments, for the narrower tubes are much more likely to become contaminated. They should be plugged with cotton and placed in a surgical package containing 25 to 30 tubes, which should then be sterilized and not opened until the set of tubes is to be used.

Fresh sterile tissue is added to each tube. A healthy, fat, adult rabbit is chosen, one that has not even a scratch or other detectable defect upon any part of its body. The animal, which must be given no food for 24 hours, is lightly etherized and bled to death from the heart by means of a sterile needle and a large bulb pipette. All hair is then removed from the abdomen and anterior thoracic walls of the animal's body by sodium sulfide solution, and the skin is prepared with the usual surgical procedures, soap and water, bichloride solution, alcohol, and finally iodine. Instruments for obtaining the kidneys should not be boiled, but required instruments are made into a set and sterilized in a glass container in the hot air sterilizer. One set is used to open the skin, another set to open the peritoneal cavity, and a fresh pair of scissors and forceps are used to remove the kidneys. All procedures should be carried out in a dust-proof room, or under a hood, the walls of which have been wiped down with bichloride solution.

After removal to a large sterile Petri dish, the capsule is stripped from the kidney, each kidney is cut into 20 to 25 pieces, and one bit of tissue placed in each sterile test-tube. This procedure must also be carried out in a dust-proof room. While removing the capsule of the kidney and cutting it into pieces, the assistant should hold the cover of the Petri dish directly over the field of operation in order to avoid dust contamination.

The poliomyelitic material which is added to each tube consists of a bit of tissue of about the same size as the bit of rabbit kidney tissue. The infected monkey should not be allowed to die, nor, in fact, to become prostrate, but should be etherized at the height of the active symptoms. After etherization, the animal should be bled, and the visceral tissues, liver, spleen, kidney, thymus, etc., removed with exactly the same technique as was used in obtaining sterile rabbit kidney. It is important to use a separate pair of forceps and scissors for each organ.

The technique for obtaining uncontaminated poliomyelitic brain tissue is as follows: The calvarium is removed with aseptic precautions, great care being taken not to injure the dura. The dura over the hemisphere opposite the one that had been injected is then seared with a red hot knife blade, and the dura is opened with sterile instruments. A portion of the cerebrum is removed with a separate set of instruments and placed in a sterile Petri dish. This material is at once taken to a dust-proof room, when it may be cut into suitable pieces and placed immediately in the tissue test-tubes.

Methods for removing bits of sterile spinal cord are very unsatisfactory. The following method has proved the most useful. The entire cord is exposed under aseptic precautions, great care being taken not to injure the dura. A portion of the cord, 3 to 4 cm. long, is tightly tied at either end with a sterile thread and the portion removed. This bit of cord with intact dura is placed in bichloride alcohol for 60 seconds, then rapidly passed through five separate washings of sterile salt solution. The dura is wiped dry with sterile gauze, seared with a narrow hot knife blade, and is then opened with sterile instruments and the cord cut into 0.5 cm. segments, each portion being put into a tissue test-tube.

When the test-tubes have been inoculated with rabbit kidney and poliomyelitic material, ascitic fluid should be added, 15 or 18 cc. being sufficient. The ascitic fluid must, of course, have been tested carefully for sterility, both for aerobic and anaerobic organisms. It should have a specific gravity of at least 1,015, be clear, free from bile, not more than 3 months old, and should be stored in the refrigerator. It should not be heated above 50°C. or filtered. Chylous or bloody ascitic fluid is not suitable. *Before adding the ascitic fluid to the inoculated test-tubes, it should be warmed to 45°C. and placed under a vacuum to remove the air.*

Complete anaerobiosis must be obtained if one is to be successful in the cultivation of the globose bodies. In our first experiments the vacuum jar was used, but the results were disappointing. The vacuum jars were either so poorly ground that they would not hold a vacuum, or, owing to defects in the glass, they cracked or exploded under the tremendous negative pressure. These difficulties were finally met by the use of the hydrogen-nitrogen jar, which utilizes the catalytic action of platinized asbestos upon hydrogen and oxygen, and removes all traces of oxygen from the jar. The details of this apparatus have already been published (3). Subsequent experiments with this jar have given most satisfactory results. The pyrogallol acid-sodium hydroxide indicator has remained unchanged in color for weeks, showing complete anaerobiosis; and the method is so rapid, clean, and simple that it seems to promise a simple satisfactory solution for the problem of anaerobic technique.

The inoculated ascitic fluid-tissue tubes, usually twenty-five to thirty tubes, are placed in the anaerobic jar and incubated for 11 to 12 days. Control tubes, inoculated with brain and other tissues, are incubated in the open air and examined daily in order to determine the presence of contaminating organisms.

After 12 days' incubation, the jar is opened, and 0.2 cc. of the ascitic fluid is removed from the bottom of each tube, without disturb-

ing the bits of brain or kidney tissue. The special pipettes devised by Gates (4) are very useful for all poliomyelitic culture work. The material is transferred directly into freshly prepared tissue tubes. 15 cc. of air-free, warmed ascitic fluid are added to each tube as before, the tubes placed in the anaerobic jar, and at once returned to the incubator. The original tubes may be placed in the refrigerator to be examined at leisure.

The second generation of ascitic fluid-tissue tubes is incubated for 12 days and the anaerobic jar is then opened. As before, immediate transfer is carried out, 0.2 cc. of the ascitic fluid near the bottom of the tube being transferred to freshly prepared tissue tubes. To a second set of tissue tubes is added 0.1 cc. of the material from the bottom of the tubes of the second generation. To Set 1, containing 0.2 cc. of transferred material, is added warmed air-free ascitic fluid as before. To Set 2, containing 0.1 cc. of the material, are added 15 cc. of semisolid medium which has been prepared as follows:

Veal infusion agar, 2 per cent, with a reaction of +0.5 to phenolphthalein, is melted, boiled, and then rapidly cooled to 50°C. Ascitic fluid, clear, straw-colored, free from bile, with a specific gravity of 1.015 or higher, is warmed to 45°C. and placed under a vacuum to remove the air. 1 part of the melted agar, cooled to 50°C., is added to 2 parts of warmed ascitic fluid, and 15 cc. of the mixture are added to each inoculated test-tube of Set 2.

The two sets of tubes are at once placed in separate anaerobic jars and incubated. The second generation of ascitic fluid tubes from which the transfer was made is placed in the refrigerator and examined on subsequent days for the presence of the globoid bodies.

The anaerobic jar containing the set of semisolid tubes (Lot 2) is incubated for 8 days, and the jar is then opened and the tubes are examined. If any of the tubes contain suspected colonies in the depths of the semisolid medium, these tiny colonies are removed by means of a capillary pipette and examined microscopically. If positive, fresh semisolid tissue tubes are made from each colony, and these tubes are placed in the anaerobic jar and incubated. If none of the tubes show visible colonies, they are all replaced in the anaerobic jar and reincubated for 8 days. If at the end of this reincubation there is no growth in the semisolid tubes, the set is discarded.

The anaerobic jar containing the ascitic fluid tubes of the third

generation, Lot 1, is incubated for 12 days, then opened, and fresh sets of ascitic fluid and semisolid tissue tubes are made exactly as described for the second generation. This process of making ascitic fluid and semisolid tubes is continued for at least five generations, after which time, if no growth has been obtained, all tubes are discarded. Every original tube inoculated is therefore a separate experiment and must be given its own number, and a detailed record must be kept of each subsequent generation of that culture. Each lot of ascitic fluid tubes, after their period of incubation, is kept in the refrigerator, so that one may subsequently return to the earlier generation in case it is necessary to pick up a positive culture that may have died out in subsequent generations.

For example, Culture 6, brain tube No. 1, after growing for two generations in semisolid medium, refused to grow further in this medium, so that it was necessary to return to the ascitic fluid tube, brain tube No. 1, third generation, from which the strain had originally been obtained, and though the growth was very scanty, and the tube had been in the refrigerator for more than a month, nevertheless a positive permanent culture was obtained.

### *Criteria for the Determination of the Globoid Bodies.*

It is necessary to have definite criteria for the determination of the globoid bodies. These have been described in the original publication by Flexner and Noguchi (1) and in later publications and need simply be summarized for convenience.

*Morphology and Staining Characteristics.*—Morphology and staining characteristics are somewhat variable factors, the variation depending not so much upon the organism as upon the observer, and, though confirmatory, they are insufficient indices in the identification of an organism.

*Characteristic Growth in Semisolid Medium.*—It is not enough to determine the presence of an organism in the ascitic fluid medium which seems to the observer to possess the characteristic morphology of the globoid bodies. Semisolid medium should be inoculated and observed daily. There should be no apparent growth in the medium for 3 or 4 days. At the end of 72 hours or later, the characteristic minute colonies begin to appear, being definitely established only at the end of 6 to 7 days. In the anaerobic jar, the line of demarcation of growth is about 1 cc. from the surface of the medium. If the semisolid tube has been incubated in the open air, however, only a few colonies will be seen about the tissue in the bottom of the tube and extending upward about 1 cc.

*Action upon Carbohydrates.*—An additional factor for the identification of the globoid bodies depends upon their inability to attack carbohydrates. Tissue tubes of ascitic dextrose broth are inoculated with a small fragment of a semi-solid culture. The broth should be sugar-free veal infusion, +0.2 to +0.3 acid to phenolphthalein. To each 5 cc. tube of broth is added an equal amount of unheated sterile ascitic fluid plus sufficient 10 per cent sterile sugar solution to give a concentration of 0.5 per cent. The hydrogen ion concentration of the medium should be approximately that of blood neutrality; namely, 7.4. Control tubes should be made both of uninoculated tubes and of tubes inoculated with various types of streptococci. The tubes should be incubated in the anaerobic jar for 7 days and the hydrogen ion concentration determined. The globoid bodies will not attack the simple sugar, whereas the control tubes of streptococci will show a marked increase in acidity.

*Results Obtained in the Cultivation of the Globoid Bodies.*

Material from eighteen poliomyelitic monkeys has been used in the attempted cultivation of the globoid bodies. For the first four experiments, the anaerobic technique used was the vacuum jar, and in each instance the experiment was unsuccessful. For all the later experiments, the platinized asbestos method was used, and far more satisfactory results were obtained.

The material from three of the monkeys was contaminated with a pure culture of streptococcus in the first generation in many or all of the tubes. This contamination was not due to faulty technique, because all control tubes were negative, but was the result of a secondary invading streptococcus which is commonly found in the blood and tissues of animals and human beings who have been prostrate and moribund for hours or days before death finally occurred.

Of the remaining eleven monkeys, the typical globoid bodies were cultivated from seven, and a total of twenty-two separate cultures or strains was obtained. The largest number of strains obtained from a single monkey was six, whereas in two instances only one strain was obtained. Besides the twenty-two strains, eleven other strains were cultivated in the ascitic fluid medium, but I was unable to transfer them to semisolid medium, and they are, therefore, not included in the total number of cultivations. The eleven strains, incompletely isolated, were derived in part from monkeys yielding completely isolated strains. Table II summarizes the successful and partially successful results of the cultivations.

TABLE II.

*Successful and Partially Successful Cultivation of the Globoid Bodies.*

Serial No.	Generation carried before discarding.	Virus cultivations in semisolid medium.			Virus cultivations in ascitic fluid medium but not transferred to semisolid medium.		
		No. of strains obtained.	Source.	Generation in which culture was obtained.	No. of strains obtained.	Source.	Generation in which culture was obtained.
1	3rd	None.			None.		
2	4th	"			"		
3	12th	"			1	Mesenteric node.	4th
4	9th	"			1	Kidney.	4th
5	10th	1	Brain.	3rd	1	Brain.	4th
6	12th	2	" 1, cord 1.	5th	1	"	4th
			Brain.	6th			
7	10th	4	"	4th	None.		
8	11th	3	"	4th	"		
9	3rd	None.			"		
10	2nd*	"			"		
11	1st*	"			"		
12	12th	5	Brain.	1, 3rd	"		
				1, 4th			
13	8th	6	" 4, spleen 2.	4th	1	Adrenal.	4th
14	7th	None.			3	Brain 2, spleen 1.	3rd
15	4th	"			1	Brain.	3rd
16	4th	"			2	"	3rd
17	1st*	"			None.		
18	4th	1	Brain.	3rd	"		
Total . . . . .		22			11		

\* All tubes contaminated with a streptococcus.

Nineteen of the completely isolated strains were obtained from brain substance, one strain was cultivated from the cervical portion of the spinal cord, and two were cultivated from the spleen. Of the eleven incompletely isolated strains, seven were cultivated from the brain, one from the kidney, one from a mesenteric node, and one from the spleen. The shortest period of time between the making of the cultures at autopsy of the monkey and definite establishment of a positive culture was 28 days, and in the third generation. The longest

period required for the definite establishment of a strain in semisolid medium was 54 days, although the organism in this case had been found in the ascitic fluid tube in the third generation on the 30th day.

In no instance was a definitely positive culture found in the first generation of tubes, but usually the organisms were present in sufficient numbers in the second generation to suggest at least that the result would eventually be positive. The globoid bodies are so small and may so readily be confused with detritus, that even a presumptive decision should not be reached unless characteristic forms are found under at least five different microscopic fields. The organisms are so few in number in the second generation that a presumptive test, though possible, is a long and tedious process. It is much simpler and more satisfactory to make subcultures of all tubes of the second generation into the semisolid medium and thus reach a positive conclusion in the succeeding generation by means of the typical growth in colony form.

#### *Occurrence of Streptococci and Other Organisms.*

The occurrence of streptococci in certain series of the cultures has been noted. More rarely other microorganisms than streptococci were cultivated instead of or with the globoid bodies. We should consider the sources and endeavor to estimate the significance of these classes of organisms. It is obvious that culture tubes which require so much manipulation will sometimes become contaminated. This condition will account for the miscellaneous bacteria sometimes encountered in the tubes, but probably not for the streptococci which have come to occupy a position of prominence, even if not of importance, with regard to the vexed question of the etiology of poliomyelitis.

The streptococci, when present in the cultures, have not, as a rule certainly, entered with the ascitic fluid or the kidney fragment or from the air. There is no reason to doubt that they have been introduced with the fragment of nervous or other tissue with which cultivation was attempted. Whether they are also to be regarded as contaminations in the broad sense is the question at issue.

A summary of all the extraneous microorganisms encountered (Table III) reveals that a large proportion was streptococci. It is of interest to have found that the tubes prepared from the liver and

kidney of the monkeys were more frequently attended by growth of streptococci, and other extraneous organisms, than those of the spleen, and the tubes of the spleen more often showed extraneous organisms than those of the brain.

In three monkeys there was a rich growth of a pure culture of streptococci in all the tubes of the first generation, the control tubes remaining sterile. In all these instances the animal had been prostrate 24 hours or longer, and in two instances had been dead several hours when the autopsy was performed. This finding is in such definite contrast with the results of the cultures prepared from the corresponding tissues of monkeys severely paralyzed but not yet prostrate and moribund or dead that it would seem to throw considerable

TABLE III.

*Summary of Contaminations with Streptococci and Other Organisms in Poliomyelitic Cultivation Experiments.*

Tissue.	No. of original culture tubes made.	No. of tubes contaminated with streptococci in 1st generation.	No. of tubes contaminated with streptococci in all later generations.	No. of tubes contaminated with organisms other than streptococci in all generations.
Brain.....	137	25	11	10
Spinal cord.....	14	0	7	0
Kidney.....	31	7	5	0
Liver.....	31	5	2	3
Spleen.....	30	6	1	2
Adrenal.....	17	3	1	1
Mesenteric node.....	19	2	0	0
Thymus.....	10	2	0	0
Pancreas.....	4	0	0	0
Total.....	293	50*	27†	16

\* Forty-six of the fifty tubes that were contaminated with streptococci in the first generation were obtained from four monkeys that had been prostrate and moribund for a considerable time before autopsy. The remaining four streptococcic contaminations in the first generation of tubes were from fourteen other monkeys, autopsied under more favorable conditions.

† An average of more than five subcultures from each original tube was made so that the total number of contaminations with streptococci in all subsequent generations (27) represents the extraneous contaminations in over 1,000 tissue culture tubes.



light on the nature of the streptococcal invasion, detected by Mathers (5), Rosenow, Towne, and Wheeler (6), and Nuzum and Herzog (7) in human beings and monkeys who have succumbed to poliomyelitic infection.

In order to obtain more light on this subject, we cultivated, by means of the technique employed for the globoid bodies, the tissues of monkeys which had succumbed in the laboratory to tuberculosis, dysentery, and other diseases, and isolated, in several instances, streptococci from the liver, kidney, spleen, and nervous tissues.

The streptococci yielded by the tissues of the moribund and dead poliomyelitic monkeys were transplanted into carbohydrate media and injected into rabbits. The fermentation reactions were those of streptococci in general and sharply distinguished them from the globoid bodies. The inoculations gave results so closely in accordance with those described by Bull (8) as not to call for restatement here.

### *Typical Cultivation Experiments.*

*Experiment A.*—February 23. *Macacus cynomolgus*. Inoculated into left cerebral hemisphere with 0.1 cc. of an N Berkefeld filtrate of a centrifuged 5 per cent suspension of glycerolated brain and spinal cord carrying mixed virus. March 4. Tremor of head, slow movements, weakness of arms. March 5. Extensive paralysis; animal unable to rise. Etherized and bled to death from the heart. The autopsy showed the presence of typical lesions of poliomyelitis.

Cultures were made as follows: brain, 8 tubes; spinal cord, 4 tubes; intervertebral ganglion, 1 tube; kidney, 3 tubes; liver, spleen, thymus, 2 tubes each. Rabbit kidney fragments from a single animal were employed, but two specimens of ascitic fluid were used. The even-numbered tubes received ascitic fluid, Lot 7, the odd-numbered tubes Lot 10. The control tubes consisted of ascitic fluid plus kidney, ascitic fluid alone, and a kidney tissue-ascitic fluid inoculated with stock culture of globoid bodies No. 973. All the tubes were placed in the hydrogen-nitrogen jar and incubated, and in addition three tubes of ascitic fluid plus kidney and brain were incubated in the open air and examined every other day. The latter series remained sterile and was discarded on the 8th day.

March 15. Hydrogen jar opened. Sodium pyrogallate solution was colorless; hence the jar had been oxygen-free. Fresh kidney tissue-ascitic tubes were re-inoculated from each of the original tubes except the thymus tube. The fragment of thymus had floated to the top, and the tube was discarded. Rabbit kidney No. 9 was used for the new tubes; and for the even-numbered tubes ascitic fluid No. 7 and in the odd-numbered tubes ascitic fluid No. 6 were used. The usual controls were added and all placed as before in the anaerobic jar.

March 17. The microscopic examination of the first generation tubes was negative throughout.

March 26. The second anaerobic jar had remained oxygen-free. In removing the tubes, spleen tube No. 20 and brain tubes Nos. 7 and 8 were broken. Microscopic examination of the remaining intact tubes gave the following result:

*Brain Tube No. 1.*—Organisms suggestive of globoid bodies.

*Spinal Cord Tubes Nos. 10 and 12, Ganglion Tube No. 13, and Spleen Tube No. 19.*—Indefinite bodies, somewhat suggestive of the globoid bodies.

*Cord Tube No. 11 and Liver Tube No. 17.*—Grossly contaminated.

March 26. Ascitic fluid-kidney tissue tubes were inoculated from the second generation, using ascitic fluid No. 13 for the odd-numbered and No. 6 for the even-numbered tubes. Anaerobic cultivation.

April 4. The jar had remained free of oxygen. Microscopic examination of the tubes gave the following result:

*Brain Tube No. 1.*—No growth.

*Brain Tube No. 5.*—Same as No. 1.

Other tubes of the third generation suggestive of globoid bodies were: brain No. 6, cord No. 12, and kidney No. 16.

April 4. Tubes of semisolid medium were inoculated and placed in the anaerobic jar.

April 10. Jar opened. All the cultures were negative except brain No. 6, cord No. 12, intervertebral ganglion No. 13, and spleen No. 19, which were merely suggestive, and cord No. 10, which contained a growth of the globoid bodies. Transplantation from the tubes which were suggestive and the one positive to fresh tubes of semisolid medium gave no result. Thus far the cultivation experiments with this specimen of virus, which had been carried through 7 or 8 weeks, resulted in three cultures of the globoid bodies which could not be developed in the fourth generation.

The ascitic fluid-kidney tissue culture of March 26 was preserved in the refrigerator for 20 days. Transplantation from it was again made into fluid and semisolid media. Brain tube No. 1 gave a positive growth which again failed to grow in the next transplantation. But by returning again to the fluid culture of March 26, a strain of brain No. 1 culture was secured which continued to grow in semisolid medium. Similarly the ascitic fluid-tissue tube of cord No. 12, made on April 14 from the mother tube of March 26, was positive and yielded a culture capable of growing in subcultures.

Thus from a total of more than 100 tubes carried over a period of more than 2 months and through five or six generations, two strains of the globoid bodies which bore subculturing were finally obtained. In each instance the organism was detected in the second generation; but patience and persistence were needed to obtain strains which would continue to grow in artificial media. All the cultivation ex-

periments were not so long, tedious, and difficult as this one; but it has been given in detail to illustrate the intricacy of the problem of the cultivation of the globoid bodies.

*Experiment B.*—April 15. *Macacus rhesus*. A cotton plug, containing 1 gm. of fresh mixed virus, was placed in the left nares, where it was allowed to remain for 16 hours. April 22. Right arm was paralyzed. April 25. Paralysis has slowly progressed; animal was almost prostrate but was bright. April 27. Extensive paralysis; complete prostration. Etherized and bled from the heart. Autopsy showed the presence of typical lesions of poliomyelitis in the cord and brain.

Cultures were made as follows: Brain, 8 tubes; kidney, 2 tubes; spleen, 3 tubes; adrenal, 2 tubes; liver, 3 tubes; mesenteric nodes, 2 tubes; and pancreas, 2 tubes; a total of 22 tubes. Three lots of ascitic fluid were used and kidney tissue from two rabbits, the same ascitic fluid being placed in every third tube and the same rabbit kidney tissue in every alternate tube. Controls were made as usual. The hydrogen-nitrogen jar was used and was satisfactory throughout the experiment.

May 8. The anaerobic jar was opened and all tubes were found to be in good condition except one liver tube, which was broken. A complete set of tissue tubes was made up, one rabbit kidney and two fresh ascitic fluids being used. These were placed in the hydrogen-nitrogen jar as before. Microscopic examination of the tubes of the first generation showed no growth in any tube.

May 20. The anaerobic jar of May 8 was opened. Examination of the tubes revealed a typical microscopic picture of the globoid bodies in brain tubes Nos. 1 and 5. One spleen tube and one liver tube were contaminated with a large Gram-positive bacillus and were discarded. Semisolid tissue culture tubes were made from each of the remaining tissue culture tubes of the second generation and placed in a hydrogen-nitrogen jar.

May 29. The anaerobic jar of May 20 was opened. A few tiny colonies were to be seen in the bottom of the following tubes: brain No. 4, brain No. 5, brain No. 6, spleen No. 12, spleen No. 13, and adrenal No. 11. Microscopic examination confirmed the presence of the globoid bodies. There was no growth in the other tubes, and they were discarded.

Fresh semisolid tissue tubes were at once made of all the suggestive semisolid cultures of the third generation and also from the ascitic fluid tubes of the second generation, which had been kept in the ice box since May 20. These tubes were all placed in the anaerobic jar as usual.

The fourth generation tubes resulted in the definite establishment of four brain strains, three from the semisolid media of May 29 and one from the ascitic fluid media of May 20. The two spleen strains were also definitely established from the spleen semisolid tubes of May 29. Adrenal tube No. 11 yielded a typical growth in the third generation, but it refused to grow in all subsequent generations and therefore cannot be included in the series.

In this experiment, therefore, a total of six positive cultures was obtained, four from the brain and two from the spleen, out of a total of twenty-two original tubes. There were two contaminations in the series, both occurring in the second generation.

*Carbohydrate Reactions of the Globoid Bodies.*

In their original publication Flexner and Noguchi (1) state that the globoid bodies have no ability to split the polysaccharides, alcohols, or even the simple hexoses. Since it is obvious that if an organ-

TABLE IV.  
*Reaction of the Globoid Bodies upon Simple Sugars.*

Strains.	Strain No.	Dextrose.		Lactose.	
		Titration to phenolphthalein in the cold.	pH	Titration to phenolphthalein in the cold.	pH
New strains.	5	0.60	7.3	0.72	7.3
	6 (Brain No. 1).	0.56	7.3	0.60	7.2
	6 (Cord " 12).	0.62	7.3	0.62	7.3
	7	0.52	7.25	0.50	7.3
	8	0.50	7.3	0.64	7.2
	12	0.64	7.2	0.68	7.2
	4 (Brain No. 3).	0.46	7.3	0.64	7.2
	4 (Spleen " 13).	0.56	7.3	0.58	7.3
Stock strains.	1281	0.56	7.2	0.48	7.3
	1328	0.52	7.2	0.60	7.2
	973	0.56	7.3	0.70	7.3
Controls. Streptococci.*	1623-2	6.6	5.3	5.6	5.6
	1556	4.56	5.8	3.1	6.0
Controls.					
Kidney + ascitic broth.		0.60	7.1	0.62	7.2
Ascitic broth alone.		0.54	7.25	0.54	7.3

Titration results are expressed in the number of cc. of 0.1 N sodium hydroxide which would be required to neutralize 100 cc. of the medium.

The hydrogen ion concentrations were done by the Henderson-Palmer colorimetric method (9).

\* Both strains of streptococci were obtained from the first generation of brain tissue tubes from poliomyelitic monkeys.

ism is unable to affect simple sugars, it will also be unable to affect higher ones, the first necessary experiment is to determine the reaction of the organism upon simple sugars.

In our experiments dextrose and lactose only were used. The medium employed is described in the paragraphs upon technique. There are so many buffer salts in an ascitic bouillon medium that the determination of acid production by titration with 0.05 N sodium hydroxide is not satisfactory. Instead, one should use one of the various methods for the determination of the hydrogen ion concentration.

Eleven strains of the globoid bodies were inoculated into the sugar media. Eight of them had been isolated within the past 3 months, and three were stock cultures that had been cultivated in the laboratory for one or more years. Controls were added to the series of two strains of streptococci which, as contaminations, had been isolated from poliomyelitic monkeys during the previous 3 months. Controls also were added of tubes containing ascitic broth plus rabbit kidney tissue and ascitic broth alone. The tubes were incubated in an anaerobic jar for 7 days, at the end of which time there was a good growth in each tube. The results of the experiment are summarized in Table IV. Thus none of the globoid bodies were able to attack the simple sugars, while the two strains of streptococci attacked them vigorously.

#### *Inoculation of Monkeys with Cultures of the Globoid Bodies.*

That the inoculation of pure cultures of the globoid bodies, even in a remote generation, will sometimes produce infection in monkeys, attended by the symptoms and specific lesions of experimental poliomyelitis, has been shown by the reports of Flexner and Noguchi (1), and Flexner, Noguchi, and Amoss (10). On the other hand, their experiments indicate that it is exceptional for the cultivated globoid bodies to exhibit definite pathogenic properties. However, the observation was made that in some animals in which a single inoculation failed to cause any symptoms, a subsequent one was followed by typical paralysis attended by the specific lesions in the central nervous organs of the infected monkeys.

The question naturally arose whether any of the cultures of the globoid bodies isolated by me possessed pathogenic properties. Eight different strains were inoculated into *Macacus rhesus* monkeys. In some instances a single injection, in others several injections were given. Three of the inoculated animals developed some degree of paralysis following intracranial or intraspinal inoculation. In one instance the culture was in the fourth, in the remaining two in the fifth generation. The other five animals did not develop suspicious symptoms. A brief recapitulation of the protocols of the three monkeys showing paralysis is given, followed by a discussion of the significance of the experiments.

*Experiment C.—Macacus rhesus.* May 19. Left intracerebral inoculation of 2 cc. of an ascitic fluid culture M.A. in the fifth generation of the globoid bodies derived from the brain of a monkey. May 25. Animal had a convulsion, following which it was ataxic and dazed. Lumbar puncture yielded a turbid fluid under pressure containing 1,400 cells, chiefly lymphocytes, and ++ globulin with 0.1 cc. May 26. Left arm protected; slight left facial palsy; ataxia. May 28. Facial paralysis marked. Following lumbar puncture brief convulsion. Cerebrospinal fluid clearing; excess of lymphocytes. May 31. Condition improved; probably will recover; etherized for cultures and histology. The autopsy revealed a cyst at the point of inoculation, containing yellow fluid. A filtrate of the local site and an emulsion of the spinal cord and medulla were inoculated into two *rhesus* monkeys, respectively; neither developed symptoms.

Cultures were prepared from the brain, kidney, liver, spleen, mesenteric node, and adrenals. All tubes were carried through three generations without contamination. The globoid bodies were isolated from one tube only, brain tube No. 2.

Sections were prepared from the medulla, cervical and lumbar regions of the spinal cord, and intervertebral ganglia. In none were any lesions characteristic of poliomyelitis found.

The globoid bodies employed for inoculation were derived from the M.A. virus, which, in its present condition, is of low virulence for monkeys. The inoculation gave rise to a local cyst, which rarely results from the intracerebral inoculations, attended by a distinct left facial palsy and weakness (?) of the left arm. It is doubtful whether the symptoms denoted experimental poliomyelitis. Probably the facial palsy was related to the cyst formation at the inoculation site; as definite paralysis did not appear in the arm, the appearance of

weakness may have been deceptive. The complete absence of histological lesions is inconsistent with the production of typical experimental poliomyelitis; although since the only developed paralysis was facial, it is always possible that exhaustive microscopic study might have revealed poliomyelitic lesions in the nucleus of the facial nerve. However, the failure of the filtrate and tissue emulsion to transmit the disease to other monkeys also speaks against the condition having been experimental poliomyelitis.

On the other hand, the injection of the globoid bodies gave rise to a marked cellular reaction in the cerebrospinal fluid, in which the predominating cells were lymphocytic. Ordinary bacteria which set up a meningitis usually produce polymorphonuclear cells. But the chief point of importance is the great difficulty and slight success attending the recovery, by cultivation, of the globoid bodies, even from the inoculated nervous tissues. Once these cultivated microorganisms readapt themselves to the condition of growth within the living body, they resist artificial cultivation as do the original tissue parasites—a point noted in Flexner and Noguchi's first communication.

*Experiment D.—Macacus rhesus.* June 9. Inoculated into the left cerebral hemisphere with 2 cc. of a lightly centrifuged unwashed sediment of a 5 day old mass culture of the globoid bodies isolated from the spleen of a monkey. The strain of the globoid bodies had passed through five generations and been under cultivation for 55 days. This animal developed convulsions, ataxia, and weakness of arms and legs. A lumbar puncture performed on June 14 yielded fluid not under pressure, containing ++ globulin 0.1 cc. and 110 mononuclear cells per c. mm. June 24. The symptoms had all diminished. The animal was given 2 cc. of a culture of the globoid bodies from the spleen intraspinally and 4 cc. intraperitoneally. The only result of this injection was to bring about a temporary increase of the ataxia. Recovery finally became complete.

While a definite reaction was obtained in this instance, there is doubt whether the symptoms really indicated the production of experimental poliomyelitis.

*Experiment E.—Macacus rhesus.* June 2. An intraspinal injection was made of 2 cc. of a mass M.A. culture in the fourth generation. June 7. The animal was somewhat ataxic and excitable, and the right arm was protected. June 18. The animal was distinctly weak and tended to fall to the left side. Etherized. The autopsy revealed a generalized tuberculosis of the viscera. A few tubercles were detected in the meninges.

The histological examination of the spinal cord, medulla, brain, and intervertebral ganglia revealed no poliomyelitic lesions. A miliary tubercle was present in the intima of a small vein in one of the ganglia.

In other words, aside from the symptoms suggestive of experimental poliomyelitis, no pathologic basis for the diagnosis could be obtained. The experiment should be regarded as negative.

If we review the results of the inoculation of monkeys with cultures of the globoid bodies, we must conclude that, while certain symptoms suggestive of poliomyelitis were sometimes produced, in no instance was the experimental disease, as determined by the presence of typical lesions in the nervous organs, actually set up. Our experiments confirm, therefore, the conclusion arrived at by Flexner and Noguchi, that it is the very exceptional cultures only which retain pathogenic power sufficient to cause infection in monkeys.

#### DISCUSSION.

The presentation of this work on the cultivation of the globoid bodies may be considered from several points of view. Perhaps the first point that should be discussed is that relating to the ultimate results achieved. The experiments show clearly, I think, that if the experimenter has suitable poliomyelitic tissues to work on and suitable samples of ascitic fluid, and if the inoculated tubes are kept under strict anaerobic conditions, and transferred at proper intervals, successful results, in some degree, will almost surely follow. In fact, the decision arrived at was to the effect that it may be possible to cultivate the globoid bodies from practically all cases from which suitable material is available. If, for example, the ascitic fluid available during the period of May 15 to June 1 had been suitable, the globoid bodies present in the fluid medium could probably have been grown in the semisolid medium. Because of this failure, I can report the cultures merely as suggestive instead of as positive. Similarly, in other experiments (Nos. 3 and 4, Table II), it is highly probable that growth in the semisolid medium would have taken place had the hydrogen-nitrogen jar been employed instead of the vacuum jar.

However, the introduction of the hydrogen-nitrogen jar has not removed the chief drawbacks of the method; namely, the personal factor of painstaking care and perseverance. At best the methods



are long, tedious, often discouraging, requiring now and again months of time, abundance of suitable material, and an exact technique in order to succeed even in one experiment. I do not consider that I have modified fundamentally the original Noguchi technique. I have devised an alternative method which seems to possess certain advantages. And yet I never succeeded in identifying the globoid bodies in the first generation, although the original investigators did so.

The chief difficulty encountered is the establishment of the strain. It would appear either that the more pathogenic of the organisms do not develop in the artificial cultures, or that when they develop they do so at the expense, as a rule, of their power to produce infection. In this respect they may be said to resemble *Treponema pallidum*, with which they share so many cultural requirements and immunological reactions, as has previously been pointed out by others (1, 11).

After having once become established and accustomed to the artificial media, the globoid bodies grow more readily, may be more easily transferred, and will survive at refrigerator temperature for months. There are certain limitations, however, beyond which they will not go. Body fluids, preferably ascitic fluid, are required for their development, and strict anaerobiosis is also essential. Furthermore, the reaction of the culture media must be at approximately blood neutrality, and indicators, such as litmus, neutral red, Andrade's, etc., inhibit their growth.

The results obtained from the cultures of organs other than nervous tissues were unsatisfactory. The great obstacles to success with them appear to be contaminating organisms, particularly the streptococcus. This common microorganism appears to be more frequently present in the general viscera than in the central nervous organs; and the cultures prepared from the kidney and liver are the ones most often developing streptococci. Apparently also the globoid bodies are present less constantly or in smaller numbers in the general viscera. Finally, despite the exsanguination of the animal, the visceral tissues always contain a certain amount of blood, which probably interferes with the development of the globoid bodies in the initial culture tubes.

Despite these adverse factors, the successful cultivation of the organ-

ism from the spleen shows that it is contained outside the nervous and in the lymphatic visceral organs. Moreover, in four other instances, the globoid bodies were cultivated from the visceral tissues in the fluid medium, although they could not be developed in the semisolid medium. These strains included one from the adrenal of the animal yielding the spleen culture, another spleen culture, one from the kidney, and one from the mesenteric lymph node of two other monkeys. The experiments are the first reported in which the globoid bodies have been cultivated from tissues other than those of the central nervous system.

The inoculation of monkeys with the cultures should be regarded as having failed to produce the experimental poliomyelitis. The circumstances surrounding the failures are in themselves instructive. Had the deductions been based merely on the clinical symptoms, they would have pointed to the induction of the infection. The ultimate criteria of experimental poliomyelitis are (1) the typical histological lesions and (2) recommunicability of the disease by inoculation of the nervous tissues of the suspected case. Since neither of these could be satisfied in the experiments, I regarded them as negative.

#### CONCLUSIONS.

The globoid bodies, identical in morphological and cultural characteristics with the organisms described by Flexner and Noguchi, have been obtained in twenty-two cultures from the tissues of seven monkeys suffering from experimental poliomyelitis.

Twenty of the strains were cultivated from the central nervous organs, all being obtained from the cerebrum except one, which was cultivated from the cervical portion of the spinal cord.

Two strains were cultivated from the spleen.

None of the cultivated strains inoculated produced typical poliomyelitis in monkeys.

The recovery of a strain of the globoid bodies from the inoculated monkey is as difficult as is the original cultivation of the organisms from animals inoculated with the ordinary virus of poliomyelitis.

Nothing in this study has served to implicate the streptococcus in the pathology of the poliomyelitic process; the streptococcus is, how-

ever, encountered as a common contaminant or secondary invader, especially in animals which have been etherized while moribund, or which had died some hours previous to the autopsy. When the infected and paralyzed animals are killed while still strong, secondary invading bacteria, including the streptococcus, tend to be absent from the tissues.

A modified, perhaps improved, but alternative method has been devised for the cultivation of the glöboid bodies and other microorganisms demanding a high degree of anaerobiosis.

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## THE ACTION OF ANTISEPTICS ON THE TOXIN OF BACILLUS WELCHII.

### A PRELIMINARY NOTE.

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The chemical sterilization of wounds has resulted in much work on the bactericidal action of the antiseptics in general use.<sup>1,2</sup> In a former paper we reported<sup>3</sup> on the solvent action of some of the chlorinated antiseptics on necrotic tissue, pus, blood clot, and plasma clot. The only evidence that these antiseptics may have a destructive action on bacterial toxins is, first, the clinical observations of Carrel and Dehelly,<sup>4</sup> who noted that in patients with infected wounds treated with hypochlorite there seemed to be an amelioration of the general symptoms which they thought might be due to a reduction in the amount of toxin absorbed, and, second, some experiments by Lumière,<sup>5</sup> who found that pus containing virulent organisms, *B. tetani*, *B. welchii*, streptococci, and staphylococci, and presumably also bacterial toxins, became innocuous after the addition of hypochlorite solution and did not cause symptoms or death in animals injected with the mixture. Control animals, injected with the untreated pus, showed typical lesions. A second series of animals was injected with candle-filtered specimens of pus before and after treatment with hypochlorite solution. The animals that received filtrate from untreated pus showed symptoms of toxemia, while those in which the filtrate from the pus-hypochlorite mixture was injected showed no toxic effect.

It seemed desirable to perform a series of experiments with a definite toxin which could be quantitatively measured and a suitable, susceptible animal as an indicator. Bull and Pritchett<sup>6</sup> have demonstrated

<sup>1</sup> Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160. Dakin, H. D., and Dunham, E. K., *ibid.*, 1917, ii, 641.

<sup>2</sup> Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

<sup>3</sup> Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

<sup>4</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 31.

<sup>5</sup> Lumière, A., *Compt. rend. Acad.*, 1916, i, 365.

<sup>6</sup> Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

a toxin for *Bacillus welchii* which fulfills all the requirements of the so called group of soluble or exotoxins. They have standardized the virulence of this toxin, and confirmed the unpublished observation of Flexner<sup>7</sup> that the pigeon is highly susceptible to the toxin and that the lesions produced in this animal are similar to those observed in human cases of gas gangrene. As wounds infected with *Bacillus welchii* are frequently encountered in military surgery today, and as the antiseptics studied are used extensively on wounds of this character, it was decided to use the toxin of Bull and Pritchett and the pigeon as a very sensitive indicator of the relative toxicity of the various toxin-antiseptic mixtures in the series of experiments recorded here. Ten experiments were performed. Comparable results were obtained in all, and the three series recorded below are in every way typical.

#### *Method.*

*Production of Toxin.*—Virulent strains of *Bacillus welchii* were grown for 18 hours in the culture medium described by Bull and Pritchett,<sup>8</sup> which, briefly, is made as follows: To 10 cc. of 0.2 per cent glucose broth are added a few fragments of sterile rabbit muscle. Inoculations are made into this medium under a layer of sterile paraffin oil and the cultures incubated in a vacuum jar from which the air has been exhausted. After incubation, the fluid is centrifuged for 20 minutes at high speed and filtered through a Berkefeld N candle. The different lots of toxin produced in this way are found to differ considerably in potency. For example, 0.3 cc. of the filtrate used in Experiment 1 contained one fatal dose of toxin, while it required 1 cc. of the filtrate used in Experiments 2 and 3 to produce a similar effect. In all cases the toxic filtrate was titrated previously to its use to determine the smallest amount which would kill, in 12 hours or less, a pigeon weighing from 300 to 400 gm., and this amount was considered as one lethal dose.

*Treatment of Toxin with Antiseptic.*—Volumes of filtrate containing the required number of fatal doses were measured into Esmarch dishes. Horse serum, inactivated at 58°C. for 1 hour, was next added to the solutions in which it was used. Sodium chloride solution,

<sup>7</sup> Flexner, S., quoted by Bull and Pritchett.<sup>8</sup>

0.9 per cent, was added to the portions requiring additional volume, and the antiseptic to be tested was added last of all. The volume used for injection was kept constant in each experiment, with the exceptions noted below. The antiseptic was allowed to remain for 5 minutes in contact with the other substances to be injected, and then the entire volume was injected into the pectoral muscles of a pigeon. Before injection, the feathers were removed from the breast and the skin was washed with alcohol. The results of Experiment 1 are shown in Table I.

TABLE I.  
*Experiment 1.*

Pigeon No	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chlorate solution.	Result.
	gm.			cc.	cc.	
1	410	1			3	Died in 12 hrs.
2	425	2	3 cc. of Dakin's solution. *			Lived.
3	425	4	3 " " " " *			"
4	425	4	3 " " " " *	1.5		"
5	410	2	3 " " phenol " †			Died in 10 hrs.
6	425	4	3 " " " " †			" " 5 "
7	240		3 " " Dakin's " *			Lived.
8	325		3 " " phenol " †			"

\* Dakin's solution titrated 0.5 per cent sodium hypochlorite concentration (made from bleaching powder).

† Phenol solution, 0.25 per cent.

*Experiment 1.*—One fatal dose of toxin killed Pigeon 1 in 12 hours. 3 cc. of Dakin's hypochlorite solution, titrating 0.5 per cent sodium hypochlorite, protected Pigeon 2 against two fatal doses, and the same amount of the solution protected Pigeon 3 against four fatal doses of toxin. That blood serum will cause hypochlorite solution to decompose is well known, and that it will reduce the effectiveness of this solution, at least as a germicide, is shown by the experiments reported by Dakin and his coworkers.<sup>1, 2</sup> 3 cc. of Dakin's hypochlorite solution protected Pigeon 4 against four fatal doses of toxin, even in the presence of 1.5 cc. of horse serum. Phenol did not exhibit

any protective action. Pigeon 5, injected with a mixture of 3 cc. of 0.25 per cent phenol and two fatal doses of toxin, died in 10 hours. Pigeon 6, receiving the same amount of phenol solution but four fatal doses of toxin, died 5 hours after inoculation. Pigeons 7 and 8, injected with the antiseptics in the same amount and concentration as employed in the toxin-antiseptic mixtures injected into the other pigeons, survived, thus demonstrating that the antiseptics themselves were not toxic and could not have explained the death of Pigeon 5 in 10 hours and that of Pigeon 6 in 5 hours.

*Experiment 2.*—The results of this experiment, recorded in Table II, confirm those obtained in Experiment 1. Because the toxin available at this time was not so potent as that used in the first experiment, it was necessary to use greater quantities for injection. In order that the total volume of the solutions to be injected should not be increased above 12 cc. and that the relative concentration of the antiseptics should be of a degree comparable with those used in Experiment 1, it was necessary to concentrate them somewhat; therefore a triple strength Dakin's hypochlorite solution, titrating 1.5 per cent sodium hypochlorite concentration, and a 1 per cent phenol solution were used. The final strength of the sodium hypochlorite and of phenol in the injected mixtures was comparable with that of those of Experiment 1, inasmuch as the dilution was considerably greater. The results were the same as those recorded for Experiment 1 in Table I.

*Experiment 3.*—In this experiment the action of chloramine-T was contrasted with that of Dakin's hypochlorite solution and of phenol. The results shown in Table III were similar to those obtained when the hypochlorite solution was used and confirm those summarized in Tables I and II. They also show that chloramine-T is able to protect pigeons against at least three fatal doses of the toxin and that its action is still demonstrable when serum is previously mixed with the toxin and the antiseptic is required to act on it as well as on the toxin.

TABLE II.  
*Experiment 2.*

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chlor-ide solution.	Result.
	gm.			cc.	cc.	
9	270	1			7	Died in 15 hrs.*
10	310	3	2 cc. of Dakin's solution. †		3	Lived.
11	310	6	2 " " " " †			"
12†	310	6	3 " " " " †	3		"
13	275	3	2 " " phenol " §		3	Died in 15 hrs.*
14	200	6	2 " " " " §			" " 15 " *
15	240		2 " " Dakin's " †		6	Lived.
16	410		2 " " phenol " §		6	"

\* Over night.

† Dakin's solution titrating 1.5 per cent sodium hypochlorite (triple strength).

‡ In this pigeon the volume injected was 12 cc., in the others 8 cc.

§ 1 per cent.

TABLE III.  
*Experiment 3.*

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chlor-ide solution.	Result.
	gm.			cc.	cc.	
17	480	1			7	Died in 12 hrs.
18	470	3	5 cc. of Dakin's solution.*			Lived.
19	430	6	2 " " " " †			"
20†	460	6	2 " " " " †	4		"
21	420	3	5 " " phenol " §			Died in 5 hrs.
22	500	3	5 " " chloramine-T "			Lived.
23†	500	3	5 " " " "	4		"
24	320		5 " " Dakin's " †		3	"
25	450		5 " " phenol " §		3	"
26	310		5 " " chloramine-T "			"

\* 0.5 per cent sodium hypochlorite titration.

† 1.73 per cent sodium hypochlorite titration.

‡ In this pigeon 12 cc. were injected, in the others 8 cc.

§ 0.25 per cent.

|| 2 per cent chloramine-T (equivalent to 0.5 per cent sodium hypochlorite).



## DISCUSSION.

From the experiments outlined above it is apparent that Dakin's hypochlorite and chloramine-T solutions will destroy the toxin produced by *Bacillus welchii*. It has seemed more precise, for experimental purposes, to make the mixtures of toxin and antiseptic *in vitro*, but from the experiments of Lumière<sup>5</sup> and the clinical observations of Carrel and Dehelly<sup>4</sup> it seems possible that these solutions may exert a similar influence when used in the treatment of infected wounds. The fact that the detoxicating action was still demonstrable when the toxin was treated with serum before the addition of the antiseptic adds to the clinical significance of these observations, because the conditions then closely simulate those encountered when the antiseptic is applied to wounds.

Phenol solutions of a final concentration of 0.25 per cent exhibited no destructive action on the toxin, and all the animals injected with a toxin-phenol mixture succumbed in the 24 hour interval following inoculation.

The control pigeons (Nos. 7 and 8, Table I; Nos. 15 and 16, Table II; and Nos. 24, 25, and 26, Table III) always survived; the antiseptic substances in the quantities and concentrations used, therefore, were not of themselves lethal.

No attempt was made to determine the maximum number of fatal doses of toxin against which a given amount and concentration of antiseptic was able to protect, nor did we go into the question of the length of time that the antiseptic and toxin must be in contact before injection in order that detoxication may occur.

The pathology of the lesions in the pigeons that died in the above experiments was substantially the same as that described by Bull and Pritchett.<sup>6</sup> In those that did not die varying grades of local edema, congestion, swelling, and discoloration of the skin and subcutaneous tissue were observed. These lesions were never marked and in no instance did they approach those observed in the birds receiving injections which resulted fatally. Those receiving phenol alone showed slightly more marked lesions than those receiving the hypochlorite alone.

In no instance did the pigeons recorded "Lived" in the tables die in the interval of observation, which was at least 1 week and in most

instances 2 weeks or longer. Any possibility of retarded deleterious effects, therefore, from the toxin-antiseptic mixtures injected, is practically excluded.

Finally, it seems desirable to add that these observations are not recorded with the purpose of advocating the use of an antiseptic in the place of the specific antitoxin produced by Bull and Pritchett.<sup>6</sup> In human surgery the antiseptic treatment of infected wounds will doubtless be combined with specific serum therapy.<sup>8</sup>

#### CONCLUSIONS.

1. Dakin's hypochlorite and chloramine-T solutions will protect pigeons against multiple fatal doses of the toxin of *Bacillus welchii* when the antiseptic and the toxin are mixed *in vitro* and allowed to stand in contact for 5 minutes before injection.

2. The detoxicating action of the solutions is demonstrable also in the presence of serum.

3. Phenol solution, 0.25 per cent, has no such action.

We take this opportunity to thank Dr. Bull and Miss Pritchett for their help and advice in the production and use of the toxin.

<sup>8</sup>A comparison of the behavior of these antiseptics enables us to distinguish two groups. In one, the antiseptic while bactericidal possesses little or no destructive action upon the products of bacterial activity; of this group phenol is an example. In the other group, the antiseptic attacks chemically not only the bacteria but also their products and by an alteration or disintegration of the molecules of the latter alters their properties and renders them inert; of this group the chlorinated antiseptics are the most striking examples. This action of these chlorinated antiseptics is to be attributed chiefly, as pointed out by Dakin, to their affinity for the amino group of the protein molecule.



## FURTHER STUDIES ON THE PROPERTIES OF PURE VACCINE VIRUS CULTIVATED IN VIVO.

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It has been shown that a sample of vaccinia virus, free from bacterial impurity and at the same time of sufficient strength for practical purposes, can be propagated in the testes of certain animals, particularly rabbits.<sup>1</sup> There were still several points, however, which seemed to require further investigation. It was not known whether or not, through continuous cultivation *in vivo* in the testicular tissues of rabbits, the biological properties of the virus would finally undergo modifications. Since the natural habitat of the virus is the skin, it is not improbable that the new medium in which it has been induced to grow might have a deleterious influence upon it. It was necessary to determine the viability of such a virus under varying conditions, that is at different temperatures, with the addition of antiseptics, etc. It was desirable also to compare the resistance and viability of the testicular strain with those of the virus propagated in the dermal tissue. These are questions not merely of purely academic interest, but of practical importance, inasmuch as the proper utilization of the new vaccine depends to a great extent upon the right understanding of its properties.

### *Scope and Mode of Experiments.*

In the present series of experiments we studied the effect of disinfectants upon the testicular vaccine virus at different temperatures, the influence of diluents under various experimental conditions, the effects of desiccation upon the virus, and the viability of dried vaccine. The samples of testicular or of skin virus employed in each instance were highly active, being capable of producing a confluent eruption

<sup>1</sup> Noguchi, H., Pure cultivation *in vivo* of vaccine virus free from bacteria, *J. Exp. Med.*, 1915, xxi, 539.

on the shaved skin of the rabbit in 1:1,000 dilution or higher. The vaccine preparations were placed in temperatures of 37°, 18°, 4°, and 0°C. Samples were taken from different test-tubes containing the virus at intervals in order to determine its strength under various conditions. During the 1st week the tests were made every 24 hours, since some of the vaccine samples which were kept at higher temperatures underwent a rapid attenuation of virulence, so that daily titration of their strength was imperative for following the course of deterioration.

The titration of the vaccine was made by applying a number of graduated concentrations of the specimen to a corresponding number of shaved areas, 10 by 20 cm., of the dorsal skin of rabbits. Special precautions were taken to have a control virus accompany the series of tests made on every animal in order to eliminate the irregular results due to individual variations in susceptibility to the vaccine virus, for without a proper control for each rabbit no accurate estimation of the vaccine effect may be made. In the present work duplicate tests were often resorted to. It was found both practical and reliable to make as many shaved areas as the dorsal and lateral surfaces of the rabbit's body permits, leaving narrow strips of hair between the shaved areas to serve as barriers, preventing the accidental overflow of one dilution to the next area. Readings of the results were made at intervals for about 8 to 10 days after inoculation. The dilutions of different samples varied according to the rapidity with which their strength diminished, but it was customary to prepare 1:1,000, 1:100, 1:50, 1:25, and 1:10 dilutions, and undiluted stock suspension. The testing of different samples after the preliminary titration was, of course, much simplified, as in a subsequent titration fewer dilutions were sufficient to estimate the strength of the virus. Strict asepsis was exercised in handling the vaccine throughout the experiments.

For comparison the regular skin vaccines were employed. These had to be put up as emulsions containing 40 to 50 per cent glycerol or 0.5 per cent phenol.

#### *Virulence and Affinities of the Testicular Vaccine Virus.*

The first point to determine was whether a strain of vaccine virus which had been propagated for successive generations during a

period of 3 years would acquire an ascending virulence for the testicular tissues while suffering a gradual loss of its affinity for the tegumentary system. In the beginning the strain which was passed on to the testicular tissue showed a lower virulence for this organ, but it also showed a correspondingly low virulence for the dermal tissue. Upon attaining a certain degree of virulence for the testes the virus manifested a parallel increase of activity for the skin, showing no disproportion between the titers for the two kinds of tissue. It may be assumed, then, that by a prolonged passage through the testes, the affinity for the skin has in no wise been diminished. The maximum titer obtained by a testicular product in rabbits was that which produced a confluent eruption on the skin of rabbits in a dilution of 1:10,000. Such specimens were not frequently obtained, and the result probably depends upon the suitability of the animal used. There are considerable individual variations in the susceptibility of the rabbit and the calf, and it is not rare to get a specimen that requires a 1:100 dilution in order to produce a confluent reaction. The titers with the rabbits averaged about 1:1,000. Of course, a specimen possessing the activity to produce a confluent reaction in a 1:100 dilution is already strong enough to insure 100 per cent of takes among primates. The individual variations in suitability for producing a testicular virus are paralleled by the susceptibility of the skin of the same animal to the vaccine virus, regardless of the mode of propagation.

It has been stated elsewhere<sup>1</sup> that a highly potential testicular vaccine can easily set up vaccinia orchitis in the rabbit in dilutions as high as 1:100,000, while on the skin the same dilution produces a few eruptions. In this respect the virus seemed to have acquired an increase in virulence for the testicle but not necessarily to have lost its dermatophilic property. It still remained to be seen whether this orchitophilic adaptation of the virus was associated also with the general increase of virulence for other internal organs and tissues. In order to determine this point we tested the emulsions of lungs, liver, spleen, kidneys, and lymph glands, removed 5 days after inoculation, of the rabbits which had been successfully inoculated intratesticularly with the testicular virus. As controls a number of rabbits were intratesticularly inoculated with unadapted virus,

which, however, caused a marked orchitis. In a second series of animals the testicular strain was used on the skin, causing the latter to produce a confluent eruption, while several animals were vaccinated with the regular skin strain to serve as controls. The results were uniformly negative, except for a few eruptions in the areas inoculated with the lymph nodes in a few instances where the virus, irrespective of whether it was of testicular or dermal origin, was given intratesticularly.

*Localization of the Vaccine Virus after Subcutaneous and Intravenous Inoculations.*

The introduction of small quantities of the testicular virus, such as 1 cc. of a 1:1,000, 1:10,000, etc., dilution, into the blood circulation or under the skin of the rabbit produced no appreciable local or general symptoms. The injection of 1 cc. of a 1:100 dilution, however, sometimes caused a local inflammation and rise in temperature on the 4th and 5th days. No general eruption was ever observed. The injection of 1 cc. of a 1:10 dilution was sometimes accompanied by a high temperature for 3 days, and, in the case of subcutaneous injection, local tumefaction and edema, but no generalized eruption. The injection of 1 cc. or more of the undiluted emulsion produced a grave illness of 3 or 4 days, with a fever lasting for 3 days. Generalized eruptions, particularly numerous on the mucous membranes of the nose, lips, mouth, and genitalia, were observed. Camus<sup>2,3,4</sup> noted a similar phenomenon with the skin vaccine.

The examinations of different tissues removed from the animals showed that in cases of intravenous inoculation of 1:10 and 1:100 dilutions, the lymph glands and sometimes, but seldom, one of the testes contained some virus, but no bilateral orchitis or marked reaction was obtained. In case of a higher dilution than 1:1,000 we occasionally demonstrated a small quantity of the virus in the lymph

<sup>2</sup> Camus, L., De la vaccine généralisée expérimentale. Conditions de sa production, *Bull. Acad. méd.*, 1916, lxxvi, 342.

<sup>3</sup> Camus, L., Réproduction de la vaccine généralisée chez le chien, *Bull. Acad. méd.*, 1916, lxxvi, 376.

<sup>4</sup> Camus, L., La vaccine généralisée expérimentale chez la génisse et chez le singe, *Bull. Acad. méd.*, 1916, lxxvi, 433.

nodes, but never in the testes or other organs. Even the injection of 1 cc. of the undiluted vaccine failed in two experiments to localize in the testes. The other organs, except the lymph glands, were negative.

The corresponding series of experiments with subcutaneous inoculation did not bring about generalized eruptions even with the strongest dose used. A small amount of the virus in the adjacent lymph nodes was occasionally demonstrated, but far less frequently than in the intravenous series.

The rabbits which received the intravenous and subcutaneous inoculations of the virus were tested for their susceptibility to a subsequent application of a powerful vaccine, both the testicular and the dermal, within several weeks. They were found to be refractory to the new vaccination, although some of them had originally received only 1 cc. of a 1:10,000 dilution. The testes of these rabbits were also insusceptible to the inoculation with a highly active virus. The immunity brought about by the intravenous or subcutaneous inoculation of the virus was altogether comparable with that conferred by a regular procedure on the skin. A detailed report of this phase of the work will be made later.

### *Viability and Resistance of the Testicular Vaccine Virus.*

Data concerning the viability and resistance of vaccine virus ought to be abundant, but one does not easily find details of a systematic investigation. From the time of Jenner the resistance of the virus to desiccation has been known, and it was proved nearly 50 years ago<sup>5</sup> that it resists the action of glycerol when the latter is used in the proper concentration. Later Umeno<sup>6</sup> and others found that phenol in a concentration below 1 per cent does not perceptibly decrease its virulence. Yet much of what was done years ago seems to have been overlooked, and it may be of sufficient interest to publish here the experimental data obtained by the writer during the past 3

<sup>5</sup> Copeman, S. M., *Vaccination. Its natural history and pathology*, London, 1899, 156.

<sup>6</sup> Personal communication from Professor S. Umeno, Director of the Imperial Institute for the Preparation of Vaccine Virus, Tokyo, Japan.



years. Naturally, the chief object of the experiments was to study the testicular vaccine virus, but in some instances, controls with the regular vaccine virus were made as far as it was possible. From the nature of the latter, however, no experiments could have been carried out to test its viability in a suspension without antiseptics such as glycerol or phenol, while it was possible to do so with the bacteria-free testicular virus.

*Survival of the Vaccine Virus in Distilled Water and Glycerolated Media, at Different Temperatures.*

April 2, 1915. Three sets of eight test-tubes each were used. After sterilization, 11.6 cc. of distilled water were added to the first tube of each set, and to the other seven tubes 11.6 cc. of 10, 20, 40, 50, 60, 80 per cent, and pure glycerol respectively. The contents of each tube were mixed with 0.4 cc. of the stock emulsion of the testicular strain, No. 948 emulsion, which had the titer of 1:1,000 (confluent). One set was placed in a refrigerator at 4°C., the second set at 18°, and the last at 37°C. 0.5 cc. was taken from each tube and tested as usual on the skin of rabbits, three or four rabbits being used in order to test the three sets (24 tubes) simultaneously. At first tests were made daily, or every other day, but later at semimonthly, monthly, or longer intervals. Tables I, II, and III give the results.

The most striking point demonstrated in the foregoing experiments is that the vaccine virus retains its virulence much longer in distilled water than in any of the glycerolated media. Pure glycerol destroyed the virus to a considerable extent in a week and completely within a month, even at a temperature of 4°C., while the virus in water remained very active after 1 year. As the concentration of glycerol diminished its destructive effect was less noticeable, and in 10 to 20 to 40 per cent the virus remained active for at least 6 months. At 18°C., the temperature of our laboratory, the virus deteriorated rapidly. The virus was killed in 5 days in pure glycerol, in 1 month in 60 to 80 per cent, in 2 months in 10 to 20 per cent glycerol, while it was still very active in water after 3 months. These differences are much more accentuated at 37°C., where the virus was no longer alive in pure glycerol after 24 hours. In 80 per cent glycerol it was avirulent in 6 days, in 60 per cent in 7 days, in 50 per cent in 9 days, and in 10 to 20 to 40 per cent in 28 days. On the other hand, in water

TABLE I.  
*Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 4°C.*

April 9, 1915.

Medium.	Days.																
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271	360
Water.....										Cf.	Cf.	Cf.	Cf.	Cf.	Cf.	++	++
10 per cent glycerol.....																+	++
20 " "										"	"	"	"	"	"	<	+
40 " "										"	"	"	"	"	"	<	+
50 " "										"	"	"	"	"	"	<	+
60 " "										"	"	"	"	++	++	+	+
80 " "										"	"	"	"	++	++	-	-
100 " "										+	<	-	-	-	-	-	-

The reactions are designated as confluent (cf.), almost confluent, + + +, + +, +, < +, < +, and finally, 1 to 3 eruptions to an area 10 by 20 cm., to which 0.5 cc. of each dilution was applied.



a trace of the virus was still present for as long as 2 months, when it still showed a few eruptions on test. The powerful vaccinicidal property of glycerol is well brought out in this set, and in any experiment bearing upon the viability of the virus this factor should be considered. The persistence of the virus in water or in 10 to 20 per cent glycerolated water at the temperature of 37°C. is remarkable and becomes important in interpreting the results in cultivation experiments.

*Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures.*

May 24, 1915. Experiments were made similar to the foregoing but with distilled water, 0.9 per cent saline solution, Ringer's solution, 50 per cent glycerol, 0.5, 1, and 2 per cent aqueous, and 50 per cent glycerolated solutions of phenol as diluents. To 19.6 cc. of each of the solutions was added 0.4 cc. of testicular virus, No. 992 emulsion. The three sets of tubes were placed at 4°, 18°, and 37°C. respectively. The results of the tests are given in Table IV.

Table IV confirms the findings of the preceding series of experiments and further shows that at 4°C. the virus was best preserved in Ringer's solution and in 0.5 and 1 per cent phenol water, being still active at the end of 1 year. In distilled water it was weaker than in saline solution, the latter being almost as good a medium as Ringer's solution. The deteriorating effect of 50 per cent glycerol was marked in this instance. The addition of phenol in 0.5 per cent did not affect the action, although 1 per cent phenol plus 50 per cent glycerol killed the virus more quickly than either of them separately. Phenol in a 2 per cent solution and agitation of the virus with an excess of chloroform for 3 hours destroyed the virus within 24 hours. The results at 18°C. and at 37°C. indicate that there is a more complete and rapid destruction of the virus with a rise of temperature. An interesting feature seems to be the longer, if not better survival of the virus in a carbolized solution (0.5 per cent water) than in Ringer's solution, saline solution, or distilled water. In the first 7 days, the activity of the virus in the latter solutions was greater than in the carbolized medium, however.



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37	

*Survival of the Vaccine Virus in Different Atmospheres.*

In order to find whether a gradual deterioration of the vaccine virus could be delayed or prevented by preserving the virus in different kinds of gases, we placed the vaccine in sealed ampules containing either hydrogen, nitrogen, oxygen, carbon dioxide, or air. For this purpose the testicular vaccine, No. 1,035 emulsion (1:1,000 titer), was diluted 10 times with sterile distilled water, the gases were passed through the vaccine, and the receptacles hermetically sealed by fusing the drawn portions with flame. The vaccine was not exposed to heat.

The appearance of the vaccine was not altered by hydrogen, nitrogen, or air, but the passage of carbon dioxide caused a clearing of the diffuse turbidity, the precipitates adhering to the wall of the container, while with oxygen, granular precipitates appeared.

Three duplicate sets placed at 4°, 18°, and 37°C. respectively were used. The results obtained after a period of 21 days are shown in Table V.

TABLE V.

*Survival of Vaccine Virus in Various Atmospheres.*

Gas.	Results after 21 days.		
	4° C.	18° C.	37° C.
Hydrogen (sealed).....	Cfl.	+++	—
Nitrogen “ .....	“	++	—
Oxygen “ .....	“	—	—
Carbon dioxide (sealed).....	“	—	—
Air (sealed).....	“	++	—
“ (open tube).....	“	+	—

The virus retained its virulence in all the gases for 3 weeks when kept at 4°C. but became avirulent at 37°C. From the results obtained at 18°C., however, it may be concluded that in sealed ampules containing hydrogen, nitrogen, or ordinary air, the virus retained its virulence somewhat better than in an open receptacle. Pure oxygen or carbon dioxide gas destroyed the virus completely at the same temperature.

### *Effect of Acid, Alkali, and Germicides upon the Vaccine Virus.*

Knowledge concerning the resistance of the vaccine virus to various substances is a prerequisite for handling it properly. In a series of experiments hydrochloric acid, sodium hydroxide, tricresol, and phenol in varying concentrations were mixed with a testicular vaccine emulsion. Tests were made on rabbits at the end of 24 hours, 4 days, and 6 days respectively at 4°C. Table VI shows the results.

The table shows that the vaccine virus is completely destroyed by sodium hydroxide added in a concentration greater than 1:200.

TABLE VI.  
*Effect of Acid, Alkali, and Germicides.*

[illegible]



while hydrochloric acid destroyed it almost completely in a corresponding concentration. On the other hand, the contaminating micrococci of the fresh skin pulp showed a different relation, resisting the action of even 1 per cent sodium hydroxide solution for at least 24 hours, while they were completely sterilized by a 0.5 per cent hydrochloric acid solution. The action of tricresol and phenol is similar, the difference being quantitative rather than qualitative. The virus resisted 0.2 per cent tricresol or 0.5 per cent phenol for many days, as did also the contaminating bacteria. In the case of phenol, and in a lesser degree tricresol, the destructive effect was comparatively more severe upon the micrococci than upon the vaccine virus. The margin was, however, narrow. A bacterial spore cannot be sterilized by a concentration which will not destroy the vaccine virus completely.

The action of iodine was next studied in various ways, because of its effectiveness as a germicide. It was employed as a local antiseptic in the form of an alcoholic solution or as Lugol's solution. In a series of experiments we made a number of dilutions of tincture of iodine by using 10 per cent ethyl alcohol water as diluent. To 0.9 cc. of each dilution, 0.1 cc. of a 1:10 dilution of a strong testicular vaccine virus was added, the mixture incubated for 1 hour at 37°C., and then tested on rabbits. It was found that the diluent alone, that is a 10 per cent alcohol water, exerted no effect. On the other hand, the mixtures containing a dilution of the tincture of iodine above 1:10,000 became avirulent. The mixture which contained a dilution of 1:100,000 gave several eruptions instead of the confluent reaction which took place when controls without iodine were used. The experiment demonstrates how destructive this element is for the vaccine virus. Lugol's solution (iodine 1 part, potassium iodide 2 parts, and water 300 parts) destroyed the virus in a 1:100 dilution but had no effect in a 1:1,000 dilution. Attempts were made to influence the course of the vaccine reaction by administering a considerable amount of Lugol's solution or sodium or potassium salts by intravenous or subcutaneous injections for several days before and after the vaccination. No effect was perceptible. The iodide salts failed to reduce the virulence of the vaccine virus even when mixed *in vitro* in a 30 per cent solution and kept 1 hour at 37°C.

Attempts were made to sterilize the vaccine virus simultaneously

with its application to the skin or at various intervals afterwards. It was found that tincture of iodine in a concentration stronger than 1:10 inhibits the development of the eruption; in 1:400 dilution, it prevented the process from being confluent. Lugol's solution used in full strength reduced but failed to check the infection. The application after 24 hours of tincture of iodine in concentrated forms did not noticeably influence the vaccine infection.

So far no visible organism has been found as the causative agent of vaccinia. From the viewpoint of classification it seemed important to study the effects of certain protoplasmic poisons on the virus. For this purpose 0.1 cc. of testicular vaccine, No. 1,062 emulsion, was mixed with 0.5 cc. of sodium oleate, sodium taurocholate, sodium glycocholate, and sodium cholate, in varying concentrations. After 1 hour at 37°C. the mixtures were tested on rabbits. It was found that all the salts destroyed the vaccine virus in a 1:10 dilution. In a 1:100 dilution a small portion of the virus still survived, while the virus in control tubes was capable of producing a confluent reaction.

### *Effect of Desiccation upon the Vaccine Virus.*

Vaccine virus is known to withstand desiccation for a long time, but more exact knowledge is desired as to its reaction to dryness, the length of time it will survive in the dry state, and how the dried vaccine virus compares with moist emulsions at different temperatures. Many microorganisms, especially those which pass the filter, resist desiccation and remain viable for a long time. Most enzymes retain their activity better in the dry than in the moist state, especially at the higher temperatures. The question becomes one of considerable importance in the case of the vaccine virus, because of the rapid deterioration which attends the moist preparation of the virus in tropical countries. If the dried vaccine proved to resist the conditions of moisture and temperature similar to those of a tropical climate better than the liquid emulsion, it would be a great advantage to preserve the vaccine virus in the dried form.

On several occasions we have dried quantities of the organ paste of testicular vaccine virus by means of a vacuum pump. After desiccation quantities were weighed, powdered, and preserved in

hermetically sealed ampules or left in an open receptacle. One set of specimens was placed at 4°C., some at 18°, and others at 37°. The controls consisted of aqueous emulsions of the undried portion of the same tissue paste.

The reduction in weight through desiccation was not uniform, and no constancy could be expected on account of the variation in degree of edema of the organs (Table VII).

TABLE VII.

*Reduction of the Weight of the Vaccine Paste through Desiccation.*

Emulsion No.	Organ.	Original weight.	Residue.
		gm.	gm.
866	Testis.	2.0	0.38
876	"	2.5	0.38
877	"	2.0	0.3
878	"	2.0	0.32
1,045	"	1.85	0.32
1,046	"	1.6	0.25

The vaccine virus did not in any instance show its original titer after desiccation. The loss of virulence, as determined by employing corresponding quantities of the dried and moist materials, amounts to half or even more of its original strength. This was unexpected, but was true with all the dried materials. The insolubility which attends the desiccation of the vaccine paste may play a part in the loss of virulence. To resuspend and dissolve the powdered material is difficult. The results obtained during a period of 18 months indicate that the dried material remains still viable, although reduced to one one-hundredth or less of the original titers, for about 12 to 18 months at 4°C. and 18°C., but becomes inert within 30 to 60 days at 37°C. The control specimens in the moist state showed similar relations.

From the above findings it is evident that the process of desiccation as carried out is considerably destructive to the vaccine virus, and that it does not protect it from the gradual deterioration due to age which takes place at different temperatures.

## SUMMARY.

1. The virulence of vaccine virus for the testicular tissues increases until its maximum is finally reached. The selective increase is not associated with any loss, reduction, or modification in its virulence for the skin. A highly potent testicular vaccine is also highly active for the skin.

2. The testicular strain of vaccine virus has no more tendency to localize in various organs than the ordinary skin strain. Both may localize in adjacent lymph nodes when introduced intravenously, subcutaneously, or intratesticularly in sufficiently large quantities, but other organs are not involved.

3. Intravenous inoculation of an excessive amount of a powerful vaccine virus (1 to 2 cc. of undiluted stock emulsion), irrespective of whether it is from the testis or the skin, will result in a generalized eruption over the entire body surface of rabbits. The eruption may be confluent on mucous membranes of the mouth, nostrils, genitalia, etc. Intratesticular or subcutaneous inoculations of the same virus fail to produce this effect.

4. Subcutaneous or intravenous introduction of much smaller quantities of the virus does not cause an appreciable local or general reaction in the rabbit. But the animals which have once received these injections become refractory to a subsequent vaccination as applied to the skin. It seems probable that an active immunity has been conferred.

5. Experiments on the viability and resistance of the testicular strain of vaccine virus indicate that the virus is best preserved when emulsified with Ringer's solution or 0.9 per cent saline solution. Distilled water, while apparently one of the best diluents, fails to keep the virus active as long as Ringer's or saline solutions. As would be expected, the lower the temperature is, the longer the virus retains its viability. At 18° or 37°C., the deterioration of the virus proceeds rapidly. However, a small part of the virus survives after many weeks' standing at 37°C.

6. Of the two most commonly employed chemical agents for the ripening (eliminating bacteria) process of the green vaccine pulp, glycerol and phenol, the latter is the less injurious. Phenol in con-

centration above 2 per cent destroys the virus within 24 hours at any temperature, but it has almost no injurious effect when used in 0.5 to 1 per cent. On the other hand, glycerol is a powerful vaccinicide. When used in full strength it destroys the virus within 24 hours, even at 4°C. In a concentration of 40 per cent, that ordinarily recommended for the ripening, the virus retains some of its virulence for about half a year at 4°C., while at higher temperatures the same concentration kills the virus within 1 to 2 months. The virus preserved in distilled water or Ringer's solution under similar temperature conditions remains more active during this period. From this it may be concluded that glycerol is not an indifferent agent, as is assumed by many, but a powerful vaccinicide when used in high concentrations. The injurious effect is markedly accelerated at 18° or 37°C.

7. The vaccine virus retains its virulence better in a sealed tube containing either hydrogen, nitrogen, or air than in an open receptacle. The virus deteriorates when placed in a sealed tube with oxygen or carbon dioxide.

8. Desiccation decreases to a considerable degree the virulence of the vaccine virus. In the dried state the virus retains its viability about as long as does the emulsion, but it is not protected from the deterioration caused by age under various conditions.

9. Iodine is a powerful disinfectant for the vaccine virus, but its sodium and potassium salts have no effect. Various bile salts destroy the vaccine virus when employed in sufficient concentration.

## SURVIVAL OF POLIOMYELITIC VIRUS IN THE BRAIN OF THE RABBIT.

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The consensus of opinion among investigators in America and Europe is that the causative microorganism of poliomyelitis is a so called filter passer. The filterable organism or virus of poliomyelitis possesses definite properties through which it may be identified, and among these the most decisive is its ability to incite experimental poliomyelitis in the monkey. In common with other filter passers, the microbic cause of poliomyelitis is very minute. It has not been detected with certainty by microscopic examination in infectious filtered fluids. There are reasons for believing that in artificial cultures the microorganism gives rise to colonies visible to the naked eye and composed of masses of minute organisms which have been called globoid bodies.<sup>1</sup> Similar globoid bodies have been detected in microscopic preparations of the nervous organs<sup>2,3</sup> and once in the circulating blood of an infected monkey.<sup>3</sup>

Another point of view, emphasized more recently, is based on the studies of Mathers, Rosenow, Nuzum, and their associates.<sup>4</sup> According to these investigators, epidemic poliomyelitis is caused by a polymorphous streptococcus, which induces paralysis and histological changes characteristic of the disease in rabbits as well as in monkeys.

<sup>1</sup> Flexner, S., and Noguchi, H., *J. Am. Med. Assn.*, 1913, lx, 362.

<sup>2</sup> Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

<sup>3</sup> Amoss, H. L., *J. Exp. Med.*, 1914, xix, 212.

<sup>4</sup> Mathers, G., *J. Am. Med. Assn.*, 1916, lxxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxxvii, 1202; *Science*, 1916, xlv, 614; *J. Am. Med. Assn.*, 1917, lxxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxxvii, 1437; 1917, lxxviii, 24.

Since the filtered virus of poliomyelitis, while highly active against monkeys, is practically without pathogenic power for rabbits, a wide difference of experimental results needs to be explained in order to bring the two points of view into harmony.

Bull,<sup>5</sup> in this laboratory, attempted to confirm the work referred to with the polymorphous streptococcus, but without success. He carried out a large series of inoculations of streptococci derived from poliomyelitic human and monkey tissues without, in a single instance, either in rabbits or monkeys, inducing clinical symptoms or pathological lesions identifiable with those of epidemic poliomyelitis. Moreover, his efforts to immunize animals with the streptococcus so as to obtain a neutralizing serum for or to protect them against infection with the filtered virus, as Rosenow claims to have done, were wholly unsuccessful. More recently, Bull has again tried, unsuccessfully, to render a monkey immune to the virus by large intravenous injections of streptococci cultivated from the brain of a poliomyelitic human case. The protocols of this experiment follow. The question, therefore, arises as to the source as well as the significance of the streptococci found not infrequently in poliomyelitis. Smillie<sup>6</sup> found that when the cultures are made from monkeys moribund and slowly dying, or from animals which have been dead some hours, streptococci are frequently present, not only in the nervous organs, but even more abundantly in the abdominal viscera. In other words, the streptococci exhibit the characters of secondary, agonal invading microorganisms. The unreported experiment of Bull with streptococci follows. *Macacus rhesus* monkeys were used.

*Monkey A.*—Apr. 25, 1917. Injected intravenously the centrifuged sediment from a 24 hour growth in 60 cc. of ascitic dextrose broth of the third generation of streptococcus obtained from human poliomyelitic brain. The monkey remained active. Apr. 27. Injected intravenously the growth from 56 cc. of the same medium, third generation of the same streptococcus, and intracerebrally the growth from 14 cc. of the same culture. The monkey became irritable, but remained active, and was normal on May 1 when another intravenous injection from 60 cc. of the third generation culture was made. May 5. A fourth injection was given.

This monkey, the serum of which was shown on May 22 to agglutinate the strain of streptococcus in a dilution of 1:4,000, was tested (a) for neutralizing action on the filtered poliomyelitic virus, and (b) for protection against an intracerebral inoculation of the same virus.

*Monkey B. Neutralization Test.*—May 22. 2 cc. of serum from Monkey A were mixed with 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic monkey cord (active virus), incubated for 2 hours at 37°C., and placed

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<sup>5</sup> Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557.

<sup>6</sup> Smillie, W. G., *J. Exp. Med.*, 1917, xxvii, 319.

over night at 4°C., then injected intracerebrally into Monkey B. May 30. Animal excited. June 1. Ataxic. June 2. Legs paralyzed; back weak. June 3. Prostrate. June 4. Died.

*Autopsy.*—Typical gross and microscopic lesions of poliomyelitis were present in the central nervous organs.

*Protection Test of Monkey A.*—May 22. After blood was withdrawn for the serum an intracerebral injection of 0.5 cc. of active virus was made. May 27. Monkey excited and ataxic. May 28. Prostrate. May 29. Died.

*Autopsy.*—Typical poliomyelitic lesions were present in the central nervous system.

#### EXPERIMENTAL.

In order to study further the relation of the filterable virus of poliomyelitis to the rabbit, with the special view of bringing out resemblances to or distinctions from the streptococcus and of determining its power of survival in the brain *in vivo*, inoculations of the virus were made into the brain of that animal. There was no expectation of inducing infection or of setting up paralysis. Bull<sup>7</sup> injected streptococci isolated from the human poliomyelitic brain intravenously into a rabbit without producing symptoms. The rabbit was etherized after 131 days. Streptococci corresponding antigenically with the strain originally injected were found to have survived in the brain. Bull also observed that local injections of streptococci from poliomyelitic tissue sometimes produce focal lesions in which the organisms survive for long periods. In other words, the polymorphous streptococcus is, under certain conditions, sufficiently adapted to the central nervous system of the rabbit to survive there, and sometimes sufficiently pathogenic to produce focal lesions in the meninges, cerebellum, medulla, cerebrum, and even in the spinal cord, and to thus induce clinical symptoms. The lesions do not, however, partake of the nature of the characteristic lesions of poliomyelitis.<sup>5</sup>

Hence, if a relation exists between the polymorphous streptococcus and the filterable virus, the latter might at least be expected to exhibit a fair degree of ability to survive in the brain of the rabbit. As the protocols which follow show, the period of survival is short.

<sup>7</sup> Unpublished experiment.



*Rabbit A.*—Nov. 21, 1916. Under ether anesthesia 0.5 cc. of a suspension of equal parts of active poliomyelitic monkey cord and isotonic salt solution was injected intracerebrally. The rabbit remained well. Dec. 14. The animal was etherized and the brain removed aseptically. There was no visible lesion at the site of inoculation. A 10 per cent suspension of the brain tissue from the region below the point of needle penetration through the skull was prepared for injection into a *Macacus rhesus* (Monkey C).

*Monkey C.*—Dec. 14, 1916. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit A, which had received the poliomyelitic virus 22 days previously. The monkey remained well.

*Rabbit B.*—Jan. 3, 1917. Injected heavy suspension of poliomyelitic virus according to the method already described. Jan. 16. Killed. There was no visible lesion at the site of inoculation. From the brain substance surrounding the site of inoculation, a 10 per cent suspension was prepared for injection into a *Macacus rhesus* (Monkey D).

*Monkey D.*—Jan. 16, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of brain at site of inoculation of Rabbit B, which had received poliomyelitic virus 12½ days previously. The monkey remained well.

*Rabbit C.*—Mar. 5, 1917. Injected intracerebrally heavy suspension of poliomyelitic virus according to the method already described. The rabbit showed no symptoms. Mar. 12. Killed. There was no visible lesion at the site of inoculation. The brain was removed aseptically and a 10 per cent suspension of the brain substance around the site of inoculation was prepared for injection into a *Macacus rhesus* (Monkey E).

*Monkey E.*—Mar. 12, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit C, which had received poliomyelitic virus 7 days previously. The monkey remained well.

*Rabbit D.*—Apr. 3, 1917. Injected intracerebrally a heavy suspension of active poliomyelitic monkey cord. The rabbit remained well. Apr. 7. Etherized and brain removed aseptically. No visible lesion at site of inoculation. A 10 per cent suspension was prepared from the brain tissue at the site of inoculation and injected intracerebrally into a *Macacus rhesus* (Monkey F).

*Monkey F.*—Apr. 7, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit D, which had received poliomyelitic virus 4 days previously. Apr. 13. Both legs paralyzed. Apr. 14. Prostrate. Apr. 15. Died.

*Autopsy.*—The central nervous organs showed macroscopic and microscopic lesions of poliomyelitis.

#### DISCUSSION AND SUMMARY.

Suspensions of the central nervous tissues of monkeys, containing the active filterable virus of poliomyelitis, may be injected into the

brain of rabbits without setting up symptoms, provided the volume of injection does not cause dangerous increased intracranial pressure.

Aside from the pressure effects which develop quickly, no other symptoms or pathological lesions are produced by the suspensions.

The active virus of poliomyelitis survives in the brain of rabbits for 4 days, as determined by tests in the monkey, into which the excised site of injection in the rabbit brain is reinoculated. It cannot be detected by this test after the expiration of 7 days.

The virus of poliomyelitis is unadapted to the rabbit, and neither induces lesions nor survives long in the central nervous organs of that animal. In this respect it differs from certain streptococci cultivated from poliomyelitic tissues.

A monkey immunized to streptococcus cultivated from human poliomyelitic nervous tissues yielded a serum which agglutinated the streptococcus in high dilution, but was without neutralizing action on the filtered virus; and the streptococcus-immune monkey was not protected against the effects of an intracerebral inoculation of the filtered virus.

The experiments recorded provide additional reasons for concluding that the streptococcus cultivated from cases of poliomyelitis differs essentially from the filterable virus and is not the microbic cause of epidemic poliomyelitis.



## METHOD FOR INTRAVENOUS INJECTION OF GUINEA PIGS.

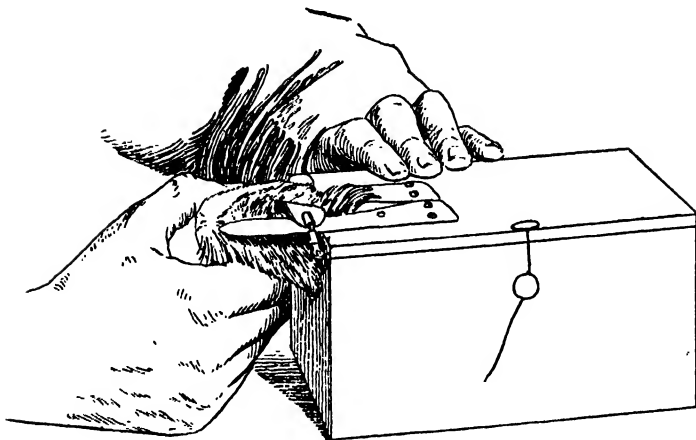
By PEYTON ROUS, M.D.

*(From the Laboratories of the Rockefeller Institute for Medical Research.)*

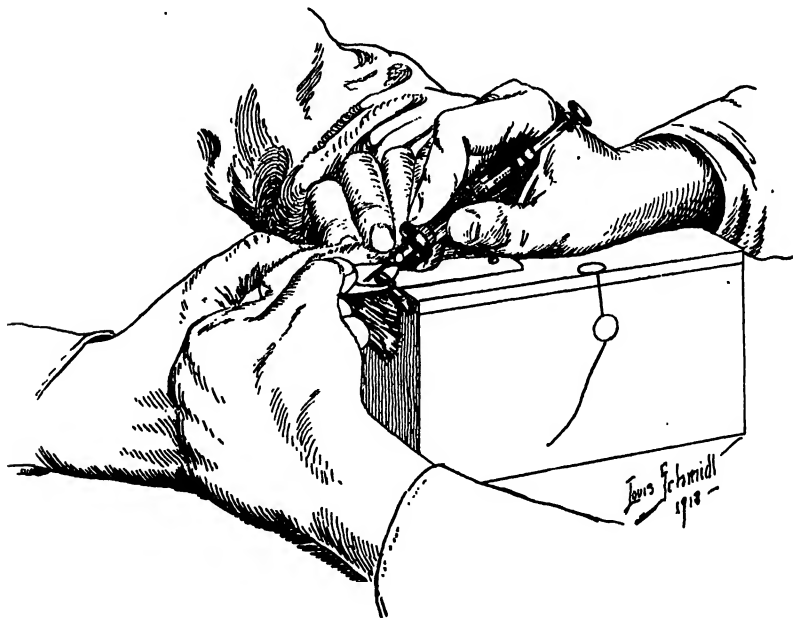
(Received for publication, January 25, 1918.)

A great drawback to guinea pigs as experimental material lies in the difficulty of intravascular injections. Direct cardiac puncture is, of course, readily performed, but much of an injected fluid may escape into the pericardial sac, or the pleural cavity, and the operator be none the wiser. Dosage is well controlled when the jugular vein is used, but this must be bared by incision, and day to day injections are well nigh impossible. To meet the requirements of such work a technique has been devised whereby repeated injections may be made into an ear vein.

Most guinea pigs have relatively large ear veins, and those near the margin are extremely superficial. They lie, indeed, so close to the surface that in the attempt to get into them with a needle the vessel is usually transfixcd or so torn that hemorrhage obscures the field. A greater obstacle to the injections is encountered in immobilizing the animal. To accomplish this, the guinea pig is placed in a small wooden box with a U-shaped opening in one end through which the head is thrust (Text-fig. 1). Extra space in the box is packed with towelling, and the lid brought down and secured, with a flexible wire, as closely as the respiration will permit. Fixed firmly to the lid are two thin plates of bone which project horizontally above and to either side of the guinea pig's head. These are platforms to which the ears may be fixed during injection. When needle and syringe are ready, the head is pressed firmly up against the platforms by an assistant, and one of the ears is drawn over and fastened lightly with a rubber-covered spring clamp (Text-fig. 1). Then the injection is made, away from the clamp, into a marginal vein (Text-figs. 2 and 3).



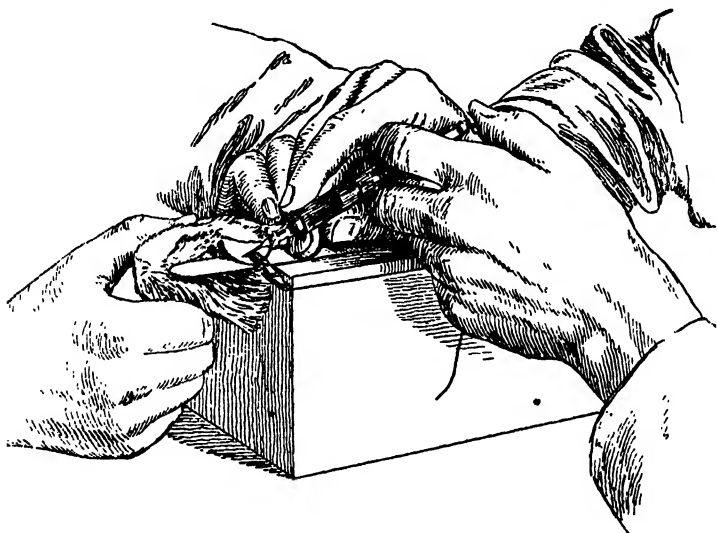
TEXT-FIG. 1. The animal's head is pressed firmly up against the platforms, to one of which the ear is fixed with a rubber-covered clamp.



TEXT-FIG. 2. With the thumb the ear is held flat and the vein distended. The bevelled side of the needle point lies against the vein as the latter is punctured.

In Text-figs. 2 and 3, the needle represented is of much larger caliber than that actually employed.

Some guinea pigs have small, indurated ears, the veins of which are not readily seen. These are unsuitable for use. Animals with black ears are a somewhat difficult material. In any instance a marginal vein should be chosen for the injection. The marginal vessels are not merely to be preferred: they are the only ones that can be used satisfactorily. The central veins, while larger, lie in a loose tissue which makes them difficult to pierce and renders leakage almost



TEXT-FIG. 3. The vein has merely been punctured, not entered further. Injection proceeds.

unavoidable. When the veins at the margin are used, any leak is at once evident: while the perivascular tissue is ordinarily so tough, that after a puncture either the injection proceeds successfully or no fluid leaves the syringe.

The needles employed should be fine (about No. 27 gauge, Brown and Sharpe), rather flexible, and at least half an inch long. Shorter ones are ordinarily so rigid as to be readily forced from the vein by any slight movement. It is important that the needle point should be bevelled obtusely. When an injection is given, the ear is held flat for the moment with the operator's thumb which by pressure distends the vein (Text-fig. 2). The needle is turned so that the

opening in it faces downward against the vessel as this is pierced. The merest puncture is sufficient. There should be no attempt to introduce the needle into the vein's lumen. All that is necessary is for an opening to be made in the vessel which will come opposite the opening in the needle. The tough perivascular tissue prevents any escape of material to either side. If the technique has been good, yet fluid fails to enter the circulation, the occurrence or absence of bleeding when the needle is withdrawn will show whether the vein has been punctured. If there is bleeding the needle may be thrust again into the little puncture wound, and now injection will often be successful. When large quantities of fluid are to be injected, the same puncture may be entered repeatedly.

The syringe should be absolutely tight and should contain no air, for the reason that successful puncture is most readily told by the slight yielding of the piston as the fluid begins to enter the vein; and this sign will be lacking when there is air in the barrel.

The technique has been described at length because strict adherence to it means success instead of failure. When it is rightly applied, injections up to 4 cc. are not difficult and may be accurately given day after day. The work is much less difficult than the injection of mice into the caudal vein, which is now so commonly practised.

## A STUDY OF THE ANTISEPTIC PROPERTIES OF CERTAIN ORGANIC COMPOUNDS.\*

By I. J. KLIGLER, PH.D.

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(Received for publication, December 20, 1917.)

Interest in the selective behavior of dyes dates back to Ehrlich's work with methylene blue. However, Stilling was probably the first to call attention to the antiseptic action of dyes on bacteria. He examined the inhibitive effect of diphenyl- and triphenylmethane dyes and recommended auramine and methyl violet as good antiseptic agents. Penzoldt tested a series of dyes and found that methyl violet, malachite green, etc., were good inhibitors. Von Drigalski and Conradi carried the idea a step further and showed that some aniline dyes inhibited *B. typhosus* less than other bacteria present in feces and recommended the use of crystal violet for the isolation of *B. typhosus* from stools, etc. Loeffler, and later Conradi, studied this phase of the problem more thoroughly, the latter testing about 400 dyes, but since they limited their observations to the action of these substances on *B. typhosus*, they contributed nothing new concerning the specific behavior of the dyes. Churchman, however, developed these observations and showed that certain violet dyes (gentian violet, crystal violet, dahlia) were more inhibitive for Gram-positive than for Gram-negative bacteria. These findings were confirmed and extended by Krumwiede and Pratt, who studied a larger series of this group of dyes.

It is evident from this brief review that the triphenylmethane dyes constitute a group of substances toxic to bacteria and reacting in a partially specific manner in the sense of Bechhold and Ehrlich. But, owing to the fact that the investigators were more absorbed in the practical application of this property, little is known concerning the nature of the action. Since my problem was similar to theirs, and since their extensive investigations resulted in only a partially successful solution, it seemed that a more effective attack might be made possible by a better understanding of the factors concerned in the specific affinity manifested by these dyes.

The noteworthy fact gathered from the literature is that all the dyes used in the isolation of *Bacillus typhosus* (crystal violet, malachite

\* Work conducted under a grant from the International Health Board of The Rockefeller Foundation.



green, brilliant green), as well as those studied by Churchman (gentian violet, crystal violet, dahlia), belong to the triphenylmethane group. Diamino or triamino triphenylcarbinol may be considered the basis of these dyes. Upon treatment with acid, these dye bases are converted into dyes themselves, the conversion, as is commonly accepted, being accompanied by a rearrangement to the quinoid form. A series of homologous dyes may be produced by substituting the hydrogen in the  $\text{NH}_2$  group by alkyl or aryl radicals. This class of substances promised, therefore, to be a good starting-point for the study of the structural chemical factors involved in the action of dyes on bacteria. A series of representative compounds was selected and their action on a number of typical bacteria studied quantitatively under carefully controlled conditions.<sup>1</sup> It soon became apparent that it would be desirable to extend the list and include for comparison a number of the simpler aromatic amino compounds. Consequently, aniline, toluidine, and some of their alkyl derivatives, and a few other related compounds were tested.<sup>2</sup>

#### *Technique.*

*Substances Used.*—A list of the substances used and their chemical constitution are given in Table I. The compounds are arranged as nearly as possible in the order of their complexity. The list is by no means exhaustive. Other compounds might have been included but were not easily obtainable, while still others were ruled out because of their insolubility.

*Method.*—The details of the method used in testing the antiseptic property of a given compound are highly important. Although comparable and fairly constant results may be obtained under identical conditions, a variation in one or another of the factors involved will cause decided fluctuations in the results. The important factors to be controlled are the composition and reaction of the medium and the condition of the culture used in the test.

<sup>1</sup> On account of the war, only a limited number of the compounds selected could be obtained.

<sup>2</sup> The dyes used were all Grüber's and were presumably fairly pure. The auramine, as well as the aniline, toluidine, and other compounds tested, was kindly furnished by Dr. W. A. Jacobs of The Rockefeller Institute for Medical Research, and were obtained from either Kahlbaum or Schuchardt.

TABLE I.  
List of Compounds Used.

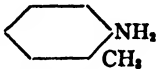
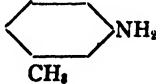

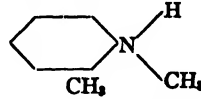
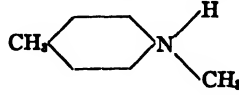
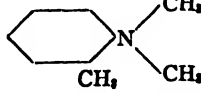
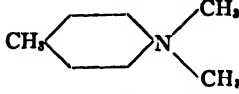
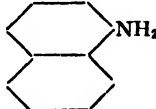
No.	Name.	Formula.
1	Methylamine (mono-).	$\text{NH}_2.\text{CH}_3.\text{HCl}$
2	" (di-).	$\text{NH}(\text{CH}_3)_2.\text{HCl}$
3	" (tri-).	$\text{N}(\text{CH}_3)_3.\text{HCl}$
4	Methyl alcohol.	$\text{CH}_3.\text{OH}$
5	Ethyl "	$\text{CH}_3.\text{CH}_2.\text{OH}$
6	Ethylamine.	$\text{NH}_2.\text{C}_2\text{H}_5$
7	Diethylamine.	$\text{NH}(\text{C}_2\text{H}_5)_2$
8	Aniline.	$\text{C}_6\text{H}_5.\text{NH}_2$
9	Methyl aniline.	$\text{C}_6\text{H}_5.\text{NH}.\text{CH}_3$
10	Dimethyl "	$\text{C}_6\text{H}_5.\text{N}(\text{CH}_3)_2$
11	Ethyl "	$\text{C}_6\text{H}_5.\text{NH}.\text{C}_2\text{H}_5$
12	Diethyl "	$\text{C}_6\text{H}_5.\text{N}(\text{C}_2\text{H}_5)_2$
13	<i>o</i> -Toluidine.	$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}_2$ 
14	<i>m</i> - "	$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}_2$ 
15	<i>p</i> - "	$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}_2$ 
16	N-Methyl <i>o</i> -toluidine.	$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}.\text{CH}_3$ 
17	N- " <i>p</i> - "	$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}.\text{CH}_3$ 
18	N-Dimethyl <i>o</i> - "	$\text{C}_6\text{H}_4.\text{CH}_3.\text{N}(\text{CH}_3)_2$ 
19	N- " <i>p</i> - "	$\text{C}_6\text{H}_4.\text{CH}_3.\text{N}(\text{CH}_3)_2$ 
20	$\alpha$ -Naphthylamine.	$\text{C}_{10}\text{H}_7.\text{NH}_2$ 

TABLE I—Continued.

No.	Name.	Formula.
21	Quinoline.	$  \begin{array}{c}  \text{CH:CH} \\  \diagup \quad \diagdown \\  \text{C}_6\text{H}_4 \quad \text{N:CH} \\  \quad \quad \quad \diagup \quad \diagdown \\  \quad \quad \text{CH}_2\text{CH}_2  \end{array}  $
22	Tetrahydroquinoline.	$  \begin{array}{c}  \text{CH}_2\text{CH}_2 \\  \diagup \quad \diagdown \\  \text{C}_6\text{H}_4 \quad \text{NHCH}_2 \\  \quad \quad \quad \diagup \quad \diagdown \\  \quad \quad \text{CH:CH}  \end{array}  $
23	Quinaldine.	$  \begin{array}{c}  \text{CH:CH} \\  \diagup \quad \diagdown \\  \text{C}_6\text{H}_4 \quad \text{N:CCH}_3  \end{array}  $
24	Auramine.	$  \begin{array}{c}  \text{CH}_3 \\  \diagup \\  \text{N} \\  \diagdown \quad \diagup \\  \text{C}_6\text{H}_{10} \quad \text{C}_6\text{H}_{10} \\  \diagdown \quad \diagup \\  \text{N} \\  \diagdown \quad \diagup \\  \text{CH}_3  \end{array}  $ <p style="text-align: center;">HN = C</p>
25	Malachite green.	$  \begin{array}{c}  \text{C}_6\text{H}_5 \\  \diagup \\  \text{C} - \text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2 \\  \diagdown \\  \text{C}_6\text{H}_4\text{:N}(\text{CH}_3)_2\text{Cl}  \end{array}  $
26	Victoria "	$  \begin{array}{c}  \text{C}_6\text{H}_5\text{Cl}_2 \\  \diagup \\  \text{C} - \text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2 \\  \diagdown \\  \text{C}_6\text{H}_4\text{:N}(\text{CH}_3)_2\text{Cl}  \end{array}  $
27	Brilliant "	$  \begin{array}{c}  \text{C}_6\text{H}_5 \\  \diagup \\  \text{C} - \text{C}_6\text{H}_4\text{N}(\text{C}_2\text{H}_5)_2 \\  \diagdown \\  \text{C}_6\text{H}_4\text{:N}(\text{C}_2\text{H}_5)_2\text{Cl}  \end{array}  $
28	Fuchsin (acid).	$  \begin{array}{c}  \text{C}_6\text{H}_5\text{CH}_2\text{NH}_2 \\  \diagup \\  \text{C} - \text{C}_6\text{H}_4\text{NH}_2 \\  \diagdown \\  \text{C}_6\text{H}_4\text{:NH}_2\text{Cl}  \end{array}  $
29	Methylviolet B.	$  \begin{array}{c}  \text{C}_6\text{H}_5\text{NHCH}_3 \\  \diagup \\  \text{C} - \text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2 \\  \diagdown \\  \text{C}_6\text{H}_4\text{:N}(\text{CH}_3)_2\text{Cl}  \end{array}  $

TABLE I—*Concluded.*

No.	Name.	Formula.
30	Crystal violet.	$\begin{array}{l} \diagup \text{C}_6\text{H}_4.\text{N}(\text{CH}_3)_2 \\ \text{C} - \text{C}_6\text{H}_4.\text{N}(\text{CH}_3)_2 \\ \diagdown \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2.\text{Cl} \end{array}$
31	Gentian "	Mixture of crystal and methyl violet plus dex trin.
32	Methyl green.	$\begin{array}{l} \diagup \text{C}_6\text{H}_4.\text{N}(\text{CH}_3)_2.\text{CH}_2\text{Cl} \\ \text{C} - \text{C}_6\text{H}_4.\text{N}(\text{CH}_3)_2 \\ \diagdown \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2.\text{Cl} \end{array}$
33	Aniline blue.	$\begin{array}{l} \diagup \text{C}_6\text{H}_3.\text{CH}_2.\text{NH}.\text{C}_6\text{H}_4 \\ \text{C} - \text{C}_6\text{H}_4.\text{NH}.\text{C}_6\text{H}_5 \\ \diagdown \text{C}_6\text{H}_4:\text{NH}.\text{C}_6\text{H}_5.\text{Cl} \end{array}$
34	Dahlia.	$\begin{array}{l} \diagup \text{C}_6\text{H}_3.\text{CH}_2.\text{NH}.\text{C}_2\text{H}_5 \\ \text{C} - \text{C}_6\text{H}_4.\text{NH}.\text{C}_2\text{H}_5 \\ \diagdown \text{C}_6\text{H}_4:\text{NH}.\text{C}_2\text{H}_5.\text{Cl} \end{array}$

*Composition of the Medium.*—That the presence of different amounts of colloidal or organic matter influences the action of dyes is well known. This is equally true of other antiseptics. An idea of the effect of different media on the antiseptic action of chemicals is obtained from the data shown in Table II. In these tests the only variable was the composition of the test media; a special peptone broth and standard beef extract broth were used. It is clear from the results that although the characteristic action of the drug is not modified by the medium, the effective concentration is appreciably altered. It is important, therefore, in testing a large number of drugs, to use a medium subject to as little variation in composition as possible. The following medium proved satisfactory:<sup>3</sup>

	per cent
Peptone.....	1.0
Potassium phosphate (dibasic).....	0.5
Sodium chloride.....	0.5
Glucose.....	0.1

<sup>3</sup>Fairchild's peptone was used throughout the investigation. The salts and sugar were chemically pure.

TABLE II.

*Action of Antiseptics in Peptone and Nutrient Broth Respectively.*

Test substance.	Dilution.	Test media.	Test cultures.*				
			2	6	17	12	14
Dibromo- $\beta$ -naphthol.	12,500	Nutrient broth.	-†	-	-	+++	+++
	25,000	" "	+	-	++	+++	+++
		Peptone "	-	-	-	+++	+++
	40,000	" "	+	+	++	+++	+++
Hexamethylenetetramine quaternary salt of <i>p</i> - chloroacetylaminotetra- ethyl <i>p'</i> , <i>p''</i> -diaminotri- phenylmethane.	15,000	Nutrient broth.	+	+	+	++	+++
		Peptone "	-	-	-	+	+
	30,000	Nutrient "	+	+	+	+++	+++
		Peptone "	-	-	-	+	++
Hexamethylenetetramine quaternary salt of chloro- acetyethylamine.	10,000	Nutrient broth.	-	-	-	+	-
	20,000	" "	+	±	±	+++	++
		Peptone "	-	-	±	++	-
	40,000	" "	+	+	+	+++	++

\* The numbers of the cultures correspond with those given in Table IV.

† - indicates no growth; ±, +, ++, +++ indicate the relative amount of turbidity at the end of 24 hours.

This culture fluid varies little, if at all, is easily prepared, and serves as a favorable substratum for all the cultures used in these tests.

*Reaction of the Medium.*—The reaction of the test medium in terms of hydrogen ion concentration is of even greater importance. This fact has been entirely overlooked until recently (Wright, 1917). A few preliminary tests showed that not only was the antiseptic power affected, but the specific behavior towards different organisms was also modified. Typical results are given in Table III. The medium given above eliminated this factor, because it had a constant pH of 7.1, the reaction usually favorable for growth of bacteria; also, since it required no adjustment, it was not subject to variation on that account.

TABLE III.

*Effect of the Reaction of the Test Medium on the Action of Antiseptics.*

Test substance.	Dilution.	Reaction of test medium.*	Test cultures.†				
			2	6	17	12	14
Caffeine.	1: 100	<i>pH</i>					
		6.2	±	±	±	+	±
		7.4	±	±	±	±	—
		8.2	±	±	±	±	—
Hexamethylenetetramine quaternary salt of <i>p</i> -chloroacetylaminoleukomalachite green.	1: 10,000	6.2	—	—	—	+++	±
		7.4	+	+	±	+++	++
		8.2	+	+	+	+++	++
Dibromo- $\beta$ -naphthol.	1: 12,500	6.2	—	—	—	++	++
		7.4	—	—	—	+++	++
		8.2	—	—	+	+++	+++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	1: 10,000	6.2	—	—	—	—	—
		7.4	—	—	—	+	—
		8.2	—	—	—	++	±

\* Nutrient broth was used in all these tests. A quantity of broth was prepared and tubed and N acid or alkali added sterily to give the desired pH.

† The cultures and symbols are the same as in Table II.

*Test Cultures.*—The condition of the test culture was the third important factor that had to be considered. Variations are likely to occur either because of lack of adaptation to the test fluid or on account of the inherent fluctuations of the organism itself. To eliminate the former, the cultures were grown in the test broth for at least 3 days, daily subcultures being made; the latter were partly controlled by using wherever possible recently isolated organisms and more than one strain of each type. The strains used, together with their origin and some descriptive remarks, are given in Table IV.

*Procedure.*—The procedure given below was followed throughout the work. The peptone broth was put up in flasks and autoclaved. 5 cc. portions were then pipetted out sterily into sterile test-tubes and incubated in a saturated incubator over night. Solutions of the substance to be tested were made up in sterile water and graded amounts added to the broth to give the desired concentration. The stock so-

TABLE IV.

Culture No.	Name.	Source.	Remarks.
123	<i>B. aerogenes</i> .	American Museum of Natural History.	Indol —; Voges-Proskauer reaction +; typical.
14	<i>B.</i> “	Isolated from stool, 1916.	Indol +; Voges-Proskauer reaction —; not typical; behaves like <i>B. aerogenes</i> .
24	<i>B. cloacæ</i> .	American Museum of Natural History.	Indol —; Voges-Proskauer reaction +; does not liquefy.
11	<i>B. coli (communis)</i> .	Isolated from stool, 1916.	Indol +; sucrose —.
13	<i>B.</i> “ “	“ “ “ 1916.	“ +; “ —.
12	<i>B.</i> “ ( <i>communior</i> ).	“ “ “ 1916.	“ +; “ +.
15	<i>B.</i> “ “	“ “ “ 1916.	“ +; “ +.
17	<i>B. typhosus</i> .	“ “ Patient O., 1916.	
19	<i>B.</i> “	Isolated from Carrier L., 1916.	Agglutinated with typhoid serum. Culturally typical.
20	<i>B.</i> “	Isolated from Carrier C., 1916.	
2	<i>B. dysenteriae</i> Flexner.	Old Institute laboratory stock.	Maltose — (Hiss-Russell).
24	<i>B.</i> “ “	Isolated by Dr. Smillie, 1916.	Maltose + (Flexner).
26	<i>B.</i> “ “	Isolated by Dr. Smillie, 1916.	Maltose — (Hiss-Russell).
6	<i>B.</i> “ Shiga.	Institute stock strain (Gay).	Reacts typically.
27	<i>B.</i> “ “	Isolated by Dr. Smillie, summer, 1916.	“ “
30	<i>B.</i> “ “	Albany stock 114 F.	“ “
21	<i>B. proteus</i> .	Isolated from stool, 1916.	
106	<i>B. subtilis</i> .	American Museum of Natural History.	
347	<i>Staphylococcus aureus</i> .	American Museum of Natural History.	

lutions were made up of such strength that no more than 1 cc. or less than 0.1 cc. had to be added to give the proper dilution. The cultures to be inoculated were filtered through sterile cotton filters, diluted with broth to give uniform turbidity, and a large standard loop was inoculated into the broth tubes. The tubes were then incu-

bated for 24 hours and the growth was recorded in terms of degree of turbidity. This gave the inhibitive power of the substance in 24 hours. In a few cases the killing power in 2 and 24 hours respectively was ascertained by subculturing the broth tubes to agar slants. This procedure was not carried out systematically because of the time consumed and since for the purposes of the study it was sufficient to determine the inhibitive property in a constant time limit.

## RESULTS

The results are given in Table V. The compounds used are arranged in the order of their increasing antiseptic power, and the results recorded in terms of the highest inhibiting dilution (first row) and the dilution which just failed to inhibit (second row). These two figures indicate the limits of the zone in which the inhibiting dilution lies. Whenever the difference between the two figures was too great, additional tests were made to reduce the gap. When more than one test was made, the average of the results was taken, and when more than one strain of a given organism was used, the average for the type is given. On the whole, individual strains of the typhoid, dysentery, or *aerogenes* bacilli varied but little, while more decided fluctuations were obtained with *Bacillus coli*. The repeated tests checked fairly closely with the original ones.

The facts brought out in Table V are difficult to summarize. In general, it is clear that on starting with aniline or its mono- or dimethyl derivatives, the introduction of a methyl group in the nucleus, as seen in the behavior of the corresponding toluidine derivatives, results in a definite increase in the inhibitive power of the compound. This is also evident from the contrast between quinoline and quinaldine. Similarly, the antiseptic property is enhanced by the substitution of either methyl or ethyl radicals in place of the hydrogens in the  $\text{NH}_2$  group. The amount of increase, up to a certain point at least, depends on the number and character of the alkyl radicals introduced. A second alkyl produces a more marked rise than the first, while an ethyl group is more effective than a methyl radical. This general phenomenon is also observed among the dyes. Beginning with fuchsin there is a progressively increasing antiseptic action which



TABLE V.  
*Inhibition of Growth of Bacteria by Certain Chemical Compounds.*

Antiseptic compound.	Gram-negative.								Gram-positive.	
	<i>B. aerogenes.</i>	<i>B. coli</i> A.	<i>B. coli</i> B.	<i>B. typhosus.</i>	<i>B. dysenteriae</i> F.	<i>B. dysenteriae</i> S.	<i>B. proteus.</i>	<i>B. subtilis.</i>	<i>S. aureus.</i>	
Methyl alcohol.	10* 20	10 20	5 10	10 20	10 20	10 20				
Ethyl alcohol.	15 25	15 25	10 20	20 25	25 40	25 40				
Aniline.	300 350	250 300	250 300	350 400	450	450	350 400	500 1,000	1,000 5,000	
<i>o</i> -Toluidine.	475 550	475 550	475 550	475 550	600 1,000	600 1,000				
<i>p</i> -Toluidine.	475 550	475 550	450 475	550 600	600 650	600 650				
Methyl aniline.	650 700	600 650	600 650	650 700	950 1,000	950 1,000	650 700	1,000 5,000	6,000 10,000	
Ethyl aniline.	1,000 1,100	1,000 1,100	1,000 1,100	1,100 1,200	1,350 1,500	1,350 1,500	1,100 1,200			
Methyl <i>o</i> -toluidine.	1,100 1,350	950 1,100	950 1,100	1,100 1,350	1,400 1,600	1,400 1,600	1,100 1,350			
Methyl <i>p</i> -toluidine.	1,100 1,350	950 1,100	950 1,100	1,100 1,350	1,400 1,600	1,400 1,600	1,100 1,350			

Dimethyl aniline.	1,350 1,500	1,350 1,500	1,350 1,500	1,500 1,750	1,750 2,000	1,750 2,000	1,100 1,350		
Dimethyl <i>o</i> -toluidine.	1,550 1,750	1,550 1,750	1,550 1,750	1,750 2,100	2,100 2,500	2,100 2,500	1,350 1,550		
Dimethyl <i>p</i> -toluidine.	2,500 2,650	2,650 2,900	2,500 2,650	2,900 3,200	3,500 5,000	3,500 5,000	2,500 2,650		
Diethyl aniline.	4,500 5,400	4,500 5,400	4,500 5,400	6,000 7,000	7,000 8,500	7,000 8,500	4,000 4,500		
Quinoline.	1,400 1,600	1,100 1,400	1,100 1,400	1,600 2,100	1,600 2,100	1,600 2,100	1,100 1,400		
Tetrahydroquinoline.	1,600 2,100	1,400 1,600	1,400 1,600	1,600 2,100	1,600 2,100	2,100 5,000	1,400 1,600		
Quinaldine.	2,600 3,400	2,100 2,600	2,100 2,600	2,600 3,400	2,600 3,400	3,400 5,000	2,100 2,600		
$\alpha$ -Naphthylamine.	2,600 3,100	2,600 3,100	2,100 2,600	2,600 3,100	3,100 7,500	3,100 7,500			
Auramine.	1,800 2,200	2,200 2,700	2,200 2,700	2,700 3,500	2,700 3,500	4,200 5,200	1,500 1,800	10,000 25,000	
Fuchsin.	8,000	8,000 12,000	12,000 15,000	12,000 15,000	100,000 110,000	110,000 200,000	12,000 150,000	300,000 500,000	
Malachite green.	20,000	40,000 45,000	45,000 50,000	30,000 35,000	250,000 500,000	500,000 1,000,000	35,000 40,000	1,000,000 2,000,000	

\* The numbers indicate dilutions; the first row the inhibiting dilution, the second the one which failed to inhibit.



runs parallel with the increase in the number of methyl or ethyl groups. The triethyl derivative is about as effective as the hexamethyl, while the tetraethyl is the most active of the series. The behavior of the *o*- and *p*-dimethyl toluidines indicates that position may be a factor in determining the degree of antiseptic action. The effect of the introduction of chlorine into the benzene nucleus is seen from the differences between malachite green and victoria green. This effect on the introduction of halogen has been observed before in other classes of compounds. It is also interesting to note that the substances containing two aromatic nuclei, namely naphthylamine, quinoline, quinaldine, and the diphenylmethane dye, auramine, are more potent than the corresponding monophenyl derivatives, whereas the triphenyl derivatives are the most active of the substances tested.

It would seem, then, that the inhibiting effect of these substances is due on the one hand to aniline with the benzene nucleus as its basis, and on the other to the number of these nuclei. The effect is consistently enhanced by the addition of alkyl radicals, either to the nucleus, or to the amino group. The number and character of these radicals also determine the degree of effectiveness.

An exception to the general phenomenon of the increase of inhibitive action produced by the increase in the number of alkyl groups is seen in the anomalous behavior of methyl green. This substance is identical with crystal violet, with the exception that one of the tertiary nitrogens of this dye has been changed as a quaternary salt by the addition of methyl chloride. Contrary to the expectation of an increase in antiseptic power, this dye is almost inert. It is also noteworthy that in the case of the triphenyl derivative of rosaniline, aniline blue, in which the hydrogens are substituted by phenyl groups, there is a decided reduction in inhibitive action.

In some respects, these results are in accord with those obtained by other workers with other classes of compounds. The well known difference between phenol and cresol and the observations of Jacobs, Heidelberger, and Bull of the progressively increasing bactericidal action, on proceeding from the dimethyl to the diethyl and dipropyl derivatives of certain quaternary salts of hexamethylenetetramine, may be cited as instances.

While it is possible to point to the factors concerned in the enhancement of the germicidal power, it is difficult to account for the specific behavior of these substances. All the compounds tested are decidedly more active against the Gram-positive than the Gram-negative bacteria. This is true as well of aniline and its methyl derivative as of auramine and the triphenylmethane dyes. It is interesting to note that while aniline and its derivatives are more active against *Staphylococcus aureus* than *Bacillus subtilis*, the converse is true of the dyes.

Partial specificity becomes most marked in the triphenylmethane dyes. This is particularly evident in their more potent action against the Gram-positive organisms and the dysentery bacilli. Though the dysentery bacilli are exceedingly sensitive to the action of these substances, they are decidedly less so than the Gram-positive bacteria. These dyes are also markedly inactive against *Bacillus aerogenes* and, with the exception of fuchsin, are more inhibitive for *Bacillus coli* than for *Bacillus typhosus*.

The fact that all these dyes behave alike, irrespective of the number and character of the alkyl radicals, indicates that the molecule as a whole is concerned with the partial specific action. This is in accord with the fact that three of these dyes have been used in the isolation of *Bacillus typhosus*.

Mention should be made of the side-light which the behavior of these organisms towards this group of substances throws on their possible relationship. *Bacillus typhosus* and *Bacillus aerogenes* (also *Bacillus paratyphosus* B) are more sensitive to the simple aniline derivatives and less so to the dyes than *Bacillus coli*. The latter, as well as *Bacillus dysenteriae*, is relatively much more sensitive to the dyes than to the aniline compounds. The extreme sensitiveness of the dysentery bacilli to the action of the dyes is especially interesting. While there was little difference between the behavior of the two classes, the Flexner cultures invariably showed a greater tolerance for fuchsin than did the Shiga bacillus. Their extreme sensitiveness to this class of chemical compounds renders it unlikely that any representative of the group may be found that will be of service in isolating them from polluted materials.

## CONCLUSIONS

This study of the inhibitive effect of aniline and some of its derivatives and of the triphenylmethane dyes on certain bacteria warrants the following tentative conclusions:

1. The composition and reaction of the medium exert a marked influence on the behavior of the antiseptic. The higher the concentration of organic nitrogenous compounds (peptone) in the medium, the lower is the effective concentration of the dye. The reaction of the medium modifies the specific action of the antiseptic, owing probably to an alteration in the bacterial cell.

2. The germicidal action of the compounds is a function of the benzene nucleus, the added elements or radicals, their number, and, in the case of the dyes, probably the quinoid structure of the nucleus.

3. As far as tested, the increase in the number of alkyl radicals increases the antiseptic power. Methyl green is an interesting exception to this rule, for the change of one of the nitrogens to the quaternary salt is accompanied by an almost complete loss in inhibitive action.

4. The antiseptic power is enhanced to a greater extent by an ethyl than a methyl group, and the second alkyl produces a proportionately greater increase than the first. It appears that the relative position of the introduced group may be a factor in determining the relative improvement in the effectiveness of the compound.

5. The introduction of a methyl group in the nucleus consistently enhances the inhibitive action of the compound and its alkyl derivatives. This is evident from a comparison of the action of aniline and its derivatives with that of toluidine and its corresponding derivatives.

6. The simple aniline derivatives, as well as the dyes, are more toxic for the Gram-positive than the Gram-negative bacteria. Of the former, *Bacillus subtilis* is more sensitive to the dyes than *Staphylococcus aureus*, while the reverse is true in the case of the aniline compounds.

7. The most marked specific selective effect is manifested by the triphenylmethane dyes. *Bacillus aerogenes* and *Bacillus typhosus* possess a higher resistance to these substances than *Bacillus coli* or

*Bacillus dysenteriae*. The last is exceedingly sensitive. This partial specificity is apparently a function of the molecule as a whole.

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## FREE ANTIGEN AND ANTIBODY CIRCULATING TO- GETHER IN LARGE AMOUNTS (HEMAGGLUTININ AND AGGLUTINOGEN IN THE BLOOD OF TRANSFUSED RABBITS).\*

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PLATES 12 AND 13.

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A singular phenomenon, hitherto undescribed, may frequently be observed in the shed blood of rabbits rendered plethoric by repeated small transfusions from compatible donors.<sup>1</sup> In fresh slide preparations the red corpuscles begin almost at once to clump into masses, and within a few moments the separation of plasma and cells is complete. The blood film, homogeneous at first to the naked eye, is transformed into a mixture of clear fluid and large red flakes. In defibrinated blood allowed to stand at room temperature, the cells fall out rapidly as a red granular sediment, which, in the course of a few hours, may become a solid mass that cannot be broken up without hemolysis. The clumping can occur *intra vasam*, as may be shown by inducing stasis in the rabbit's ear with a tourniquet applied at the base. When the marginal ear vein is opened after  $\frac{1}{2}$  hour of such stasis, the blood flowing forth is seen to consist of numerous rather large, dark red flakes in a clear fluid. Under ordinary conditions, the clumping is plainly an extravascular phenomenon, being first

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<sup>1</sup> The method of transfusion has already been described (Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1917, xxv, 665). The rabbits received intravenously, 6 days in 7, 10 cc. of whole citrated rabbit blood obtained by the cardiac aspiration of compatible donors. For each recipient a series of donors were employed in rotation. Their compatibility had been determined by the method of Rous and Turner, *J. Am. Med. Assn.*, 1915, lxiv, 1980.



visible in slide preparations some 12 to 40 seconds after the blood is drawn.

*Protection of the Organism.*

The clumping of the red cells would result in pulmonary emboli and be quickly fatal, were it not in some way prevented *in vivo*. Our first tests had to do with this phenomenon. The problem presented proved unexpectedly easy of solution. The clumping is absolutely conditioned by temperature. If no precautions are taken to keep warm the rabbit's ear when bound with a tourniquet, clumping occurs in the vessels, as already described. But if the bound ear is kept in water at the body temperature, the blood taken from it after half an hour shows no clumping, and this appears only secondarily as cooling occurs. Again, if a little fresh blood is taken into each of two thick-walled glass tubes of capillary bore, one of which has been chilled in the ice box, the other heated to body temperature, a gross clumping will be seen almost at once in the cold tube, whereas in the warm one the blood remains homogeneous during the 3 or 4 minutes before clotting takes place. Blood allowed to drop directly into a few cubic centimeters of cold salt solution shows clumping almost before it can be distributed in the medium; whereas if the solution has the body temperature, the corpuscles remain separate for 24 hours even—the longest time over which we have observed them. If, after 24 hours of warmth, the mixture is cooled in running water, clumping occurs in case the initial dilution of the blood has not been too great.

*The Clumping Is a True Agglutination.*

The character of the temperature control suggests that the clumping is caused by hemagglutinins. Landsteiner has shown that the isoagglutinins and the weak normal autoagglutinins of several animal species, among them the rabbit, are similarly governed by temperature and have most effect in the cold.<sup>2</sup> The clumping, viewed microscopically, has certainly the appearance of a true agglutination, while the circumstances of its occurrence are such as would favor the development of agglutinins. It is most pronounced when the rabbit

<sup>2</sup> Landsteiner, K., *Munch. med. Woch.*, 1903, 1, 1812.

has received from ten to fifteen transfusions. After the first five or six, a change begins to be noticeable in the shed blood. Rouleau formation is more marked than normally. Then, as the injections are continued, the rouleaux of the shed blood, which are strikingly long and perfect, tend to draw together into masses in which they undergo little disorganization (Figs. 1 and 2). Finally, the tendency to clumping becomes so strong that the rouleaux present when the blood is first shed collapse after a few moments into irregular agglomerates of corpuscles lying against one another without definite arrangement. In slide preparations these masses are at first connected by large trunks of cells, but shrinkage soon takes place—doubtless from closer apposition of the cells—and the trunks pull out into thin strands, often only one or two cells thick (Figs. 3 and 4). Within the large plasma spaces thus opened up, there are almost no corpuscles, red or white. When pressure is put on the cover-glass the cells are often stretched into long ropes, but they hold together tenaciously as if made of a sticky, elastic material. When the pressure is released they resume their normal shape.

If the clumping is caused by a true agglutinin, a separation of this element from the cells should be possible. It has been accomplished by repeatedly washing the cells in warm salt solution in which, as already stated, they are not clumped. Cells thus washed in the centrifuge remain unclumped when sedimented and cooled. But they at once clump when placed in serum obtained from a specimen of the blood defibrinated and centrifuged in the warm.

Attempts were now made to obtain the agglutinin in salt solution by the method Landsteiner employed with the weak, normal auto-agglutinin of rabbits.<sup>2</sup> Landsteiner allowed a small quantity of cells in a large quantity of serum to stand over night on ice. Complete agglutination took place. The cell mass was now washed several times in ice cold salt solution and then placed in a little of the fluid at body temperature. After some hours it was centrifuged while still warm. The heating had liberated the agglutinin, which passed into the salt solution, and the latter now possessed the ability to agglutinate cells.

In the case of our transfused rabbits the serum factor responsible for clumping was so strong that there was no necessity for the serum

to preponderate greatly over the cells or for more than a brief chilling and warming.

*Experiment 1.*—A little of the blood of a transfused rabbit was taken into a test-tube surrounded by a water jacket at 38°C. and was defibrinated with glass beads. From this 0.6 cc. was pipetted off and cooled in ice. The corpuscles rapidly clumped and fell to the bottom of the tube. After 45 minutes centrifugation was done in an ice jacket. The serum was immediately pipetted off and kept. It will be termed Serum A. The cells, which had formed a solid mass, were now twice washed with 3.5 cc. of ice cold salt solution. The cell mass showed no tendency to break up when thus handled. All fluid was now pipetted away, 0.3 cc. of fresh salt solution put on, and the tube transferred to a water bath at 40°C. Within 5 minutes the mass had broken up into a homogeneous cell suspension. After 10 minutes more, an attempt was made to throw down the cells while still warm, but though a warm water jacket was used, the temperature fell sufficiently during the process for some clumping to occur. The tube was therefore warmed again for 15 minutes and again centrifuged rapidly, but now in a jacket of warm paraffin oil. This time the heat was retained, no clumping occurred, and the fluid—Fluid B, as it will be called—was immediately taken off for test. The cell sediment was then twice washed in an excess of warm salt solution and made up to the original blood bulk. The cells remained unclumped.

The following mixtures were now set up:

- (a) 1 part cell suspension + 3 parts Fluid B.
- (b) 1 part cell suspension + 3 parts Fluid B + 9 parts salt solution.
- (c) 1 part cell suspension + 3 parts Serum A + 9 parts salt solution.
- (d) 1 part cell suspension + 12 parts salt solution.

In (a) marked clumping of the cells took place almost at once at room temperature. The other three mixtures were cooled in ice. Strong clumping was observed after a few minutes in (b), slight clumping in (c), and none at all in (d). From the presence of a slight clumping in the mixture (c) it is evident that the factor responsible for agglutination was not completely removed from the serum when the defibrinated blood was cooled to 0° C.

### *Variation with Temperature.*

In experiments such as the foregoing, success was obtained only after the necessity for careful maintenance of the essential temperatures had been recognized. During the separation of the agglutinin a moment's accidental cooling or warming was sufficient to fix or liberate it in large part from the cells, thus leading to confusion. When blood was defibrinated in the warm and then gradually cooled in tubes that permitted of microscopic inspection, slight agglutination was

found to appear as the temperature fell from 36° to 35°C. At 33°C. large clumps formed; and at room temperature (22°) the agglutination was massive. When the tube was warmed again, all clumping disappeared at between 35° and 36°C. In the experiment for the separation of agglutinin given in detail above, all agglutinin was not absorbed from the serum at 0°C. This may have been due to insufficient contact of serum and cells owing to the rapid clumping and sedimentation of the latter when suddenly chilled. We have repeatedly noted that a potent serum, if allowed to separate from the clot at room temperature, may contain no agglutinin whatever.

### *Reversibility of the Reaction.*

The rapid variation in the clumping with changes in temperature has led us to investigate the reversibility of the agglutination. A sample of blood was defibrinated in the warm, as usual, filtered through gauze, and placed, first on ice, and, when clumping was complete, in water at body temperature. This was repeated as fast as massive clumping or its reverse, complete dissociation, had occurred. After nine coolings and warmings, the cells still clumped and separated as rapidly and completely as at first. There was an entire absence of the gummy change seen when cells are repeatedly clumped by a heteroagglutinin.<sup>3</sup>

### *Strength of the Agglutinin.*

The great variation in the clumping at different temperatures and the rapidity with which the agglutinating principle is fixed or freed has rendered difficult a precise determination of its strength. We have employed a crude method, allowing the blood to fall from the rabbit's ear, drop by drop, into known quantities of warm salt solution, and noting the agglutination when the mixtures have been cooled for some minutes at room temperature. Under these circumstances the dilution of both antigen and antibody vary, but they vary alike, maintaining practically a constant relation to each other. There is not the same likelihood of error in the ingredients as when mixtures are made of cells and serum separated from each other in the warm.

<sup>3</sup> Landsteiner, K., and Reich, M., *Centr. Bakteriöl., 1te Abt., Orig.*, 1905, xxxix, 83.

But the temperature of the salt solution must be above 37°C., since even slight cooling results in some clumping of the cells before they can be properly distributed.

The strongest agglutinin found in the transfused rabbits caused well defined clumping in a mixture of one drop of blood with 100 cc. of salt solution; that is, in a plasma dilution of approximately 1:2,800.<sup>4</sup> No clumping occurred in the 1:5,600 mixture. The plasma of a second rabbit agglutinated the cells when diluted 500 times. These were exceptional instances. In the majority of cases the plasma failed to clump the cells when it was diluted with more than 20 parts of salt solution.

The clumping phenomenon did not regularly appear in transfused rabbits. Indeed, in ten out of twenty transfused with a special view to its development it was never observed despite the fact that the transfusions were continued far beyond the usual period. Furthermore, in rabbits transfused persistently any agglutinating factor that had developed tended to disappear. A similar disappearance of precipitin following unduly prolonged immunization has been recorded by Tchistovitch<sup>5</sup> and Nuttall.<sup>6</sup> Sudden reductions in the plethora of the recipients, accomplished by bleeding, failed to induce or increase the clumping phenomenon, as did also a use of donors with cells agglutinable by the recipient's plasma.

### *Agglutination and Anemia.*

The peculiar temperature control of the agglutination in the transfused animals has an obvious, if superficial, likeness to that occurring in paroxysmal hemoglobinuria. And the fact that the animals with the strongest agglutinin developed a sudden anemia, in the midst, so

<sup>4</sup> The percentage volume of the blood plasma was reckoned from a comparison of the rabbit's hemoglobin with that of normal rabbits of which the cell plasma ratio had been established with Epstein's hematocrit. In these normal animals the cells averaged 42 per cent and the plasma 58 per cent of the blood volume. Twenty drops of blood were assumed to make 1 cc. This was the case in actual tests.

<sup>5</sup> Tchistovitch, T., *Ann. Inst. Pasteur*, 1899, xiii, 406.

<sup>6</sup> Nuttall, G. H. F., *Blood immunity and blood relationship*, Cambridge, 1904, 127.

to speak, of their plethora, has in this respect a special interest. The hemoglobin of the rabbit with an agglutinin active in a 1:2,800 serum dilution fell after the fifteenth transfusion from 128 (Sahli) to 75 per cent in the course of 4 days, despite the injection on each of these days of the usual 10 cc. of blood. The transfusions were now stopped, and the hemoglobin fell to 37 per cent in 2 days more, after which a gradual recovery ensued. The animal at no time manifested symptoms of distress. Some of the blood changes in this rabbit and others of like sort have already been described by Robertson in another connection.<sup>7</sup> In these instances the hemagglutinin was at its greatest strength when the anemia developed, while in animals with a weak agglutinin or none, an anemia was never observed, but, on the contrary, plethora was maintained for weeks after the transfusions had been stopped.

No adequate search has yet been made for an hemolysin in the plasma of the animals becoming anemic, but we have chilled, without result, two plethoric rabbits possessing a weak agglutinin (active in a 1:5 dilution of the whole blood) in the hope of initiating a drop in the hemoglobin. The chilling was accomplished by means of ice cold water, in which the well shaved ear of the rabbit was submerged for  $\frac{1}{2}$  to 1 hour. Throughout this period the circulation in the cold ear was exceptionally good. The rectal temperature fell to 37°C., considerably below the normal for the rabbit, but not low enough to produce the *in vitro* agglutination of blood corpuscles.

### *Persistence of the Agglutinin.*

The agglutinating principle, once it has appeared in a blood, persists for a long period and is relatively uninfluenced by the disappearance of plethora, or by sudden intercurrent anemia of the sort just described, or by moderate bleedings. In a rabbit, for example, in which the hemoglobin fell from 125 to 27 per cent in the course of a few days, with a gradual return to the normal of 90 per cent, the agglutinin persisted throughout. 110 days after the normal hemoglobin had been finally reached, the blood still showed clumping when di-

<sup>7</sup> Robertson, O. H., *J. Exp. Med.*, 1917, xxvi, 221.

luted with two volumes of salt solution. This was 133 days after the last transfusion.

#### SUMMARY.

In rabbits transfused almost daily with the whole citrated blood of other rabbits, an extraordinary condition often develops, which manifests itself in an almost immediate clumping together of all the red cells in specimens of the shed blood. This clumping is due to one or more true agglutinins, of which the strength may be such as to cause clumping in a 1:2,800 plasma dilution.

The agglutinating principle circulates with the corpuscles against which it is effective; but under ordinary circumstances intravascular clumping fails to occur because the union of antigen and antibody can take place only at a temperature several degrees below that of the body. If the temperature is sufficiently lowered, as when a tourniquet is applied to the rabbit's ear, intravascular clumping ensues. In defibrinated blood, gradually cooled, clumping is first noted as the temperature of 35°C. is approached; and at room temperature (22°) the corpuscles will often come together in a short time into a single, solid mass. At 0°C. the agglutination is still more marked. The reaction seems to be completely reversible, for when the blood is warmed again, the clumps break up and disappear at between 35° and 36°C. Cooling and warming with the resultant clumping and dissociation can be carried out many times on the same blood specimen without apparent change in the corpuscles or in the rapidity of the reaction. The response to temperature changes is extremely prompt.

Once it has been elicited, the agglutinating principle may persist for a long time after the transfusions are stopped. In one instance it was still strong 133 days after the last transfusion. During this period the plethora was succeeded by a severe anemia, which in turn was recovered from. In many rabbits no agglutinin develops, and a continuance of the transfusions will not elicit it. Indeed, when present it tends to disappear if the transfusions are persisted in.

In several of the animals in which the agglutinin was strongest, the plethora was suddenly succeeded by severe anemia, despite continued transfusions. The character of the temperature control of

the agglutination, which somewhat resembles that of the hemolysin in paroxysmal hemoglobinuria, has led us to consider whether the blood destruction might not be due to accidental chilling of the animal. Efforts to induce a fall in the hemoglobin by placing the rabbit's ear in ice water have as yet been unsuccessful. Thus far no adequate search for an hemolysin has been made.

The object of the present paper has been to describe a condition in which large amounts of free antigen and antibody circulate together in the organism, and to demonstrate the factor which prevents their union, the results of which could easily be fatal. The causes of the condition will be dealt with in a subsequent communication.

#### EXPLANATION OF PLATES.

##### PLATE 12.

FIG. 1. A weak clumping phenomenon. The rouleaux are largely intact

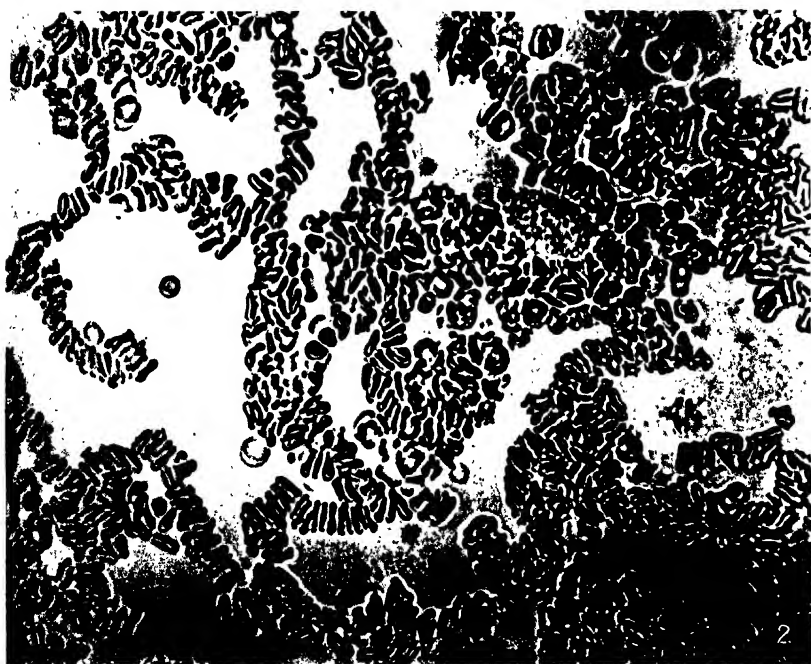
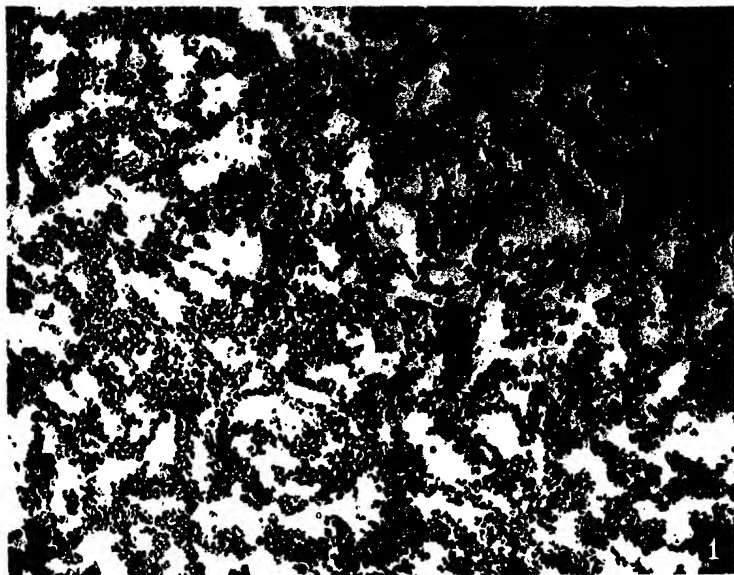
FIG. 2. A weak clumping phenomenon. Marked rouleau formation.

##### PLATE 13.

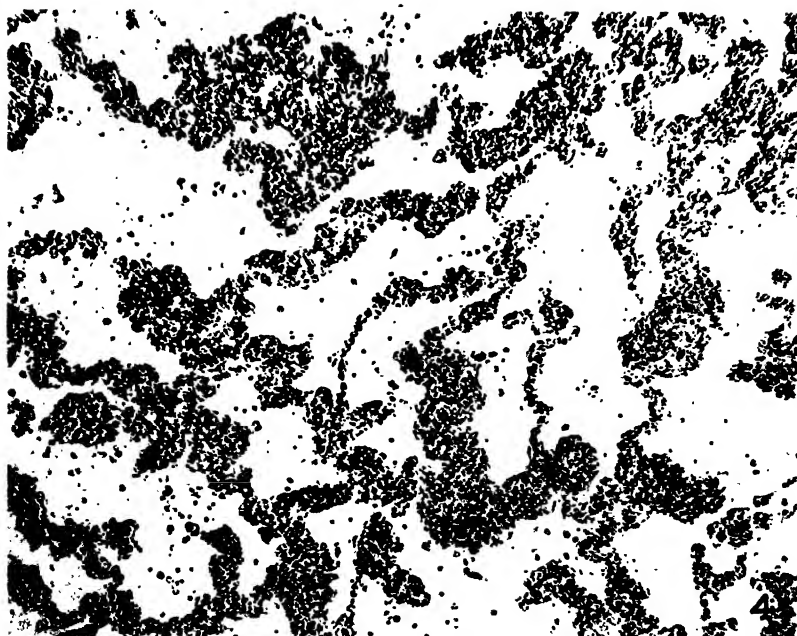
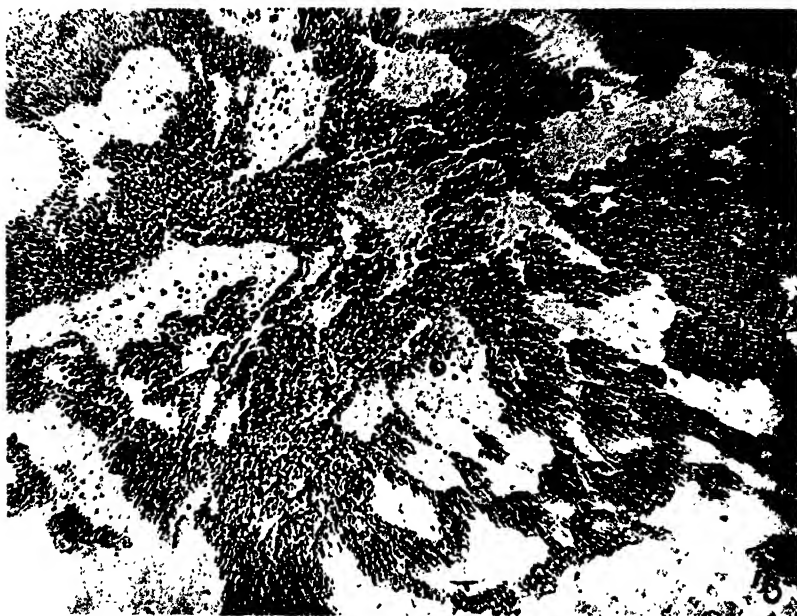
FIGS. 3 and 4. The clumping phenomenon in pronounced form. In the large serum spaces there are almost no free cells.











(Rous and Robertson: Free antigen and antibody.)



## A STUDY OF ACUTE MERCURIC CHLORIDE INTOXICATIONS IN THE DOG WITH SPECIAL REFERENCE TO THE KIDNEY INJURY.\*

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PLATES 14 TO 16.

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A review of the relatively scant literature dealing with acute mercuric chloride intoxications shows a preponderance of clinical papers with suggestions relative to the treatment of poisoning and but few investigations which have as their object an understanding of the cause of the remote tissue changes, especially those of the kidney. The investigations which have been primarily concerned with the acute pathology induced by mercuric chloride in organs remote from the intestine have either considered the injury to be dependent upon the action of the metal on the vascular tissues of the organ, or to be due to the action of the metal as such on the parenchyma of the organ during elimination.

Von Mehring<sup>1</sup> considered the toxic action of mercury in the kidney and also in the intestine to be due to a general vasomotor paralysis. Heineke<sup>2</sup> and Kaufmann<sup>3</sup> held the opinion that mercury in the blood had a thromboplastic action and that the damage to the kidney depended upon the formation of thrombi with the production of infarcts. Schmiedeberg<sup>4</sup> considers the changes in the

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<sup>1</sup> von Mehring, J., Ueber die Wirkungen des Quecksilbers auf den thierischen Organismus, *Arch. exp. Path. u. Pharm.*, 1881, xiii, 86.

<sup>2</sup> Heineke, W., Die Fermentintoxication und deren Beziehung zur Sublimat- und Leuchtgasvergiftung, *Deutsch. Arch. klin. Med.*, 1888, xlii, 147.

<sup>3</sup> Kaufmann, E., Neuer Beitrag zur Sublimatintoxication nebst Bemerkungen über die Sublimatnieren, *Virchows Arch. path. Anat.*, 1889, cxvii, 227.

<sup>4</sup> Schmiedeberg, O., *Grundriss der Pharmakologie in Bezug auf Arzneimittel-lehre und Toxikologie*, Leipsic, 5th edition, 1906.

mucous membrane of the colon and kidney to be due to the destructive action of the metal during its elimination. In a recent study of acute mercury poisoning by Burmeister and McNally<sup>5</sup> the same conclusion is apparently reached as that held by Schmiedeberg concerning the way in which the toxic effect of mercury is induced. The authors note the marked variation in the toxic action of the metal and consider that the hepatic changes vary with the duration of the intoxication, while the kidney damage varies with the size of the dose as well as the duration of the intoxication. In the animals receiving massive doses immediate renal changes develop which vary with the size of the dose, while with smaller doses the renal changes depend upon the length of time the animal is able to withstand the intoxication.

In an investigation<sup>6</sup> conducted several years ago, in which various nephrotoxic agents were employed, a study was made of the relative affinity of different kidney poisons for the epithelium of the kidney and of the relation between the degree of epithelial damage and the ability of the kidney to form urine. Mercuric chloride was used in eight animals. The salt was given subcutaneously in the dose of 10 mg. per kilo. The nephropathy induced by these injections was variable in both the frequency with which it occurred and the constancy of the pathological changes in the kidney. Two of the animals became rapidly anuric and showed an extensive necrosis of the renal epithelium, especially that of the convoluted tubules. The remaining animals either showed no toxic effect from the injections, or after a short period of albuminuria made a complete recovery.

Recent studies<sup>7,8</sup> of the acute nephropathy induced in dogs by uranium have shown a similar variation in the toxicity of this metal for the kidney. These studies have furthermore shown that when the functional capacity of the kidney has been reduced by uranium the vascular mechanism retains its responsiveness to various peripherally acting stimuli comparable in degree with that of the normal organ. The lack of functional response has been associated with a variable amount of degeneration of the renal epithelium.

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<sup>5</sup> Burmeister, W. H., and McNally, W. D., Acute mercury poisoning. A parallel histological and chemical study of the renal and hepatic tissue changes as compared with the rapidity of absorption and the amount of mercury present in the circulating blood at the time such changes occur, *J. Med. Research*, 1917, xxxvi, 87.

<sup>6</sup> MacNider, W. deB., A study of the renal epithelium in various types of acute experimental nephritis and of the relation which exists between the epithelial changes and the total output of urine, *J. Med. Research*, 1912, xxi, 79.

<sup>7</sup> MacNider, A study of the action of various diuretics in uranium nephritis, *J. Pharm. and Exp. Therap.*, 1911-12, iii, 423.

<sup>8</sup> MacNider, The vascular response of the kidney in acute uranium nephritis; the influence of the vascular response on diuresis, *J. Pharm. and Exp. Therap.*, 1914-15, vi, 123.

In later papers,<sup>9,10</sup> the observation was made that in acute uranium intoxications the severity of the epithelial damage in the kidney shows a correlation with the degree of acid intoxication induced by the metal, and, furthermore, that the damage to the kidney may in large measure be prevented by the intravenous injection of an alkaline solution.

With these observations in mind the present study of the toxicity of mercuric chloride has been undertaken with the object of ascertaining the cause of the kidney damage and with the hope that some therapeutic agent might be developed that would protect the kidney against the toxic effect of this metal. A preliminary note of this work has recently appeared.<sup>11</sup>

#### EXPERIMENTAL.

Dogs were used in the experiments which furnish a basis for this study. The animals were placed in metabolism cages, given 500 cc. of water daily by stomach tube, and fed on bread with a small amount of cooked meat. The urine was collected from the cages twice a day. Females were catheterized at the second collection of urine in order to obtain an accurate record of the total output in a 24 hour period. Forty-three of the fifty-six animals used were females. The animals were kept under observation for 3 days prior to administering the mercury. During this normal period (Table I) and following the use of the mercury the animals were studied as follows: Hydrogen ion determinations of the blood were made by the indicator method of Levy, Rowntree, and Marriott.<sup>12</sup> The alkali reserve of the blood and determinations of the carbon dioxide tension of alveolar air were made

<sup>9</sup> MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

<sup>10</sup> MacNider, The efficiency of various diuretics in the acutely nephropathic kidney, protected and unprotected by sodium carbonate. II, *J. Exp. Med.*, 1917, xxvi, 19.

<sup>11</sup> MacNider, A study of the acid-base equilibrium of the blood in acute bichloride intoxications, *Proc. Soc. Exp. Biol. and Med.*, 1917, xiv, 140.

<sup>12</sup> Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen-ion concentration of the blood, *Arch. Int. Med.*, 1915, xvi, 389.



by the methods of Marriott.<sup>13, 14</sup> The blood urea determinations were made by the method of Marshall,<sup>15</sup> following the modification suggested by Van Slyke and Cullen.<sup>16</sup> The phenolsulfonephthalein test for kidney function was conducted according to the technique devised by Rowntree and Geraghty.<sup>17</sup> Quantitative albumin determinations were made by Esbach's method, and the percentage of glucose in the urine was estimated by Benedict's reagent. The quantitative determinations of acetone were made by Folin's<sup>18</sup> method as modified by Hart.<sup>19</sup> During the course of the experiment it became necessary to ascertain the relation, if any existed, between the elimination of mercury by the kidney and the development of the acute kidney injury. In the absence of satisfactory quantitative tests the recently devised qualitative test of Elliott was used.<sup>20</sup> The test is both simple and very delicate, and may be employed in a relatively quantitative fashion by noting the amount of mercury deposited on gold leaf as an amalgam from a series of urines.

In order to induce an experimental condition comparable with that obtained when mercuric chloride is taken accidentally or for suicidal purposes, the poison was introduced by a stomach tube. The animals were first given hypodermically 0.25 cc. of a 4 per cent solution of morphine sulfate. After the initial excitement and emesis induced by the morphine, the animals became partially narcotized. During

<sup>13</sup> Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

<sup>14</sup> Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxi, 1594.

<sup>15</sup> Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

<sup>16</sup> Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

<sup>17</sup> Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909-10, i, 579.

<sup>18</sup> Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

<sup>19</sup> Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

<sup>20</sup> Elliott, J. A., A new and delicate method for the detection of mercury, *J. Am. Med. Assn.*, 1917, lxxiii, 1693.

this period of narcotization the animals were given 15 mg. of mercuric chloride per kilo. A 1 per cent solution was used. At the time of administration the dose of mercuric chloride was made up to a volume of 100 cc. with distilled water. As a result of the depressed irritability of the animals and the dilution of the dose of the poison, the irritant effect of the mercury was sufficiently modified to prevent vomiting in the majority of the animals for a period of 4 hours. Eight of the animals vomited the mercury within an hour after its use. The rest of the animals retained the poison for 4 hours or longer. Excluding the eight animals referred to above, the poison was unquestionably retained long enough for it to be absorbed and induce its remote toxic effect.

All the animals receiving mercury developed a gastroenteritis which varied widely in severity and duration. This variation apparently depended upon the total amount of the poison received by the animal and the length of time which elapsed between the administration of the poison and the commencement of vomiting. An analysis of the experiments, from the standpoint of the final outcome of the intoxicated animals, permits their classification into four groups. The first group is represented by eight animals (Table II, Group I). The animals of this group developed an intense gastroenteritis which was characterized by persistent vomiting of large quantities of fluid and by frequent, bloody, mucous stools. The animals died in a state of collapse within 48 hours after receiving the poison.

The second group of animals (Table III, Group II) also showed a severe local reaction from the mercury. The vomiting and stools were frequent. In this series the gastroenteritis showed a tendency to subside during the first 3 days of the intoxication. All the animals died within 7 days from the commencement of the intoxication. Six died in convulsions. The remaining four animals died in air-hunger.

The third group of animals (Table IV, Group III) showed a moderately severe gastroenteritis. During the subsidence of the gastroenteritis, or several days after the symptoms of this condition had disappeared, the animals showed a beginning acid intoxication. The hydrogen ion determinations of the blood were variable. The reserve alkali of the blood, however, showed a depletion and the tension of alveolar air carbon dioxide was reduced. These changes, indicative

of a beginning acid intoxication, persisted from 1 to 8 days. The twenty animals forming this group made a complete recovery in as far as any immediate effect from the mercuric chloride intoxication was concerned.

The remaining group of eighteen animals (Table V, Group IV) showed a gastroenteritis which was variable both in severity and duration. In all the animals this symptom of poisoning disappeared. Following the subsidence of the enteritis, and in three of the experiments as late as the 9th day, the animals either gradually or rapidly developed an acid intoxication, and, depending upon the severity and duration of this intoxication, became anuric. Seven of the animals died in air-hunger. From the foregoing outline of the variation in the toxicity of mercuric chloride as shown by the animals comprising the different groups, it becomes necessary to analyze the effect of the poison in the various groups with the object of ascertaining the cause of the variation and the way in which the toxic action of the metal is induced.

### *Observations on Normal Animals.*

The following observations on normal animals extended over a period of 3 days. The results have been recorded in Table I. The number of the experiment in this table corresponds with the number

TABLE I.

### *Observations on Normal Animals.*

Group No.	Experiment No.	Urine.	Albumin, glucose, acetone.	Phenolsulfonphthalein.	Blood urea.	pH.	R. pH.	Carbon-dioxide tension
		cc.		per cent	per cent			mm.
I	1	418	0	69	0.012	7.35	8.05	45
I	2	330	0	83	0.021	7.35	8.1	45
I	3	381	0	79	0.013	7.35	8.1	43
II	4	681	0	81	0.014	7.5	8.1	44
II	5	492	0	80	0.012	7.55	8.0	42
II	6	891	0	73	0.012	7.45	8.1	43
III	7	271	0	84	0.013	7.3	8.05	41
III	8	508	0	82	0.012	7.45	8.1	45
III	9	320	0	86	0.012	7.35	8.0	42
IV	10	381	0	71	0.012	7.45	8.1	42

of the animal after it had received the poison. The observations on the various groups of intoxicated animals will be found in Tables II to V. A study of the normal findings contained in Table I shows that all the animals were freely diuretic, and that the urine was free from albumin, glucose, and acetone bodies. The centrifugalized urines did not show casts. The appearance of phenolsulfonephthalein in the urine was not delayed longer than 10 minutes. The total output of the dye in a 2 hour period varied between a minimum output of 69 per cent and a maximum output of 86 per cent. The percentage of blood urea in all the animals remained very constant, varying between 0.012 and 0.021 per cent. The hydrogen ion concentration of the dialyzed whole blood prior to aeration varied between 7.35 and 7.55. After the aeration of the dialysate the reserve alkali showed slight variation, 8 to 8.1. The determinations of the tension of alveolar air carbon dioxide showed a constancy with that of the reserve alkali determinations. In as far as the urine, renal function, and acid-base equilibrium of the animals were investigated, the animals of all the groups were normal.

*Observations on the Different Groups of Animals Intoxicated by  
Mercuric Chloride.*

*Group I.*

The first group of experiments (Table II) is represented by the animals which died from the intoxication within 48 hours after receiving the mercury. All the animals showed a clinical condition comparable with the state of shock and collapse which is obtained in man from the use of a concentrated corrosive poison. They were unable to stand. The surface of the body was cold, the tongue and gums were cyanotic, the respirations shallow, and the heart beat was fast with feeble heart sounds. The pupils in four of the animals were widely dilated for several hours before death. The animals of this group had an intense gastroenteritis, as was indicated by the persistent vomiting of fluid, in two instances streaked with blood, and by the frequent, bloody, mucous stools. The autopsies showed the usual effect of a strong corrosive on the mucous membrane of the stomach and intestine.

TABLE II.  
Group I. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfone-mercury in urine.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools	Vomit.	Condition of animal.
1	15	1	790 cc.	0 gm.	0 per cent	0 mg.	75 per cent	Tr.	0.012	7.45	8.05	42 mm.	Severe enteritis.	Frequent.	Bad.
		2	0	0	0	0	0	0	0.027	7.35	8.0	38	No change.	No change.	Died in collapse.
2	15	1	70	Tr.	Tr.	0	77	Tr.	0.040	7.35	8.0	39	Severe enteritis.	Frequent.	"
3	15	1	130	"	0.39	4.612	65	"	0.061	7.3	7.9	37	"	"	"

A review of Table II, containing representative experiments from this group, shows that the output of urine is rapidly reduced. This reduction is not associated with a comparably great reduction in the elimination of phenolsulfonephthalein. There is no marked retention of blood urea. In Experiment 2, Table II, the animal formed only 70 cc. of urine in the first 24 hour period after receiving the mercury. The elimination of phenolsulfonephthalein was 77 per cent. In Experiment 3 the animal formed 130 cc. of urine in a similar period with a phenolsulfonephthalein elimination of 65 per cent. There is in this group a lack of correlation between the ability of the kidney to form urine and to secrete phenolsulfonephthalein. The urine has been either free from albumin or has contained a mere trace. In only one animal was glucose present in the urine. The urine of this animal also showed a trace of acetone, 4.6120 mg. per 100 cc. In this group of animals only a slight disturbance in the acid-base equilibrium of the blood occurred. In Experiment 2, Table II, at the end of the first 24 hours of the intoxication the alkali reserve was 8 as compared with a normal reading of 8.1, and the tension of alveolar air carbon dioxide was 39 mm. as compared with the normal of 45 mm. The animal died of shock 6 hours later. Experiment 3, Table II, shows the greatest disturbance in the acid-base equilibrium of any of the members of the group. Associated with the reduction in the reserve alkali of the blood the elimination of phenolsulfonephthalein is reduced from a normal of 75 per cent to 65, and both glucose and acetone appear in the urine. At this early stage of the intoxication only a trace of mercury was found in the urine.

The animals comprising Group I die in collapse which is apparently dependent upon the severity of the local corrosive action of the mercury in the stomach and intestine. Death occurs before sufficient time has elapsed for the development of the kidney injury. The reduction in the output of urine is probably dependent upon a disturbance in the functional capacity of the vascular mechanism of the kidney induced by the deflection of arterial blood away from the kidney to the splanchnic viscera. The absence of degenerative changes in the kidney and the presence of an intense congestion of the splanchnic vessels would apparently permit this deduction.

*Pathology of the Kidney.*—The kidneys of this group of animals

were removed immediately after death. Postmortem changes were eliminated. The kidneys have had a dark cyanotic appearance. On section the organs have shown an engorgement with venous blood which has been especially marked at the corticomedullary boundary zone and has extended into the medulla in the form of streaks outlining the return veins from the venous arches. Histologically the glomerular vessels have shown an engorgement with blood. No exudate or free hemorrhage has been observed in the glomeruli. The endothelial nuclei of the glomerular capillaries have appeared prominent and stained deeply. The epithelium of the tubules, especially that of the convoluted tubules, has shown an early cloudy swelling, consisting in the appearance of albuminous granules in the cytoplasm without much increase in the volume of the cells. The nuclei of the epithelium have shown an increase in size out of proportion to the changes in the size of the cells. No exudate or extravasation of blood has been observed in the intertubular connective tissue. (Fig. 1.)

### *Group II.*

The second group of experiments consists of the animals which first developed a severe gastroenteritis, and then, during the subsidence of the gastroenteritis, developed an acute acid intoxication with an associated anuria. The animals of this group either died in air-hunger or in convulsions.

A review of the experiments representative of this group of animals (Table III) shows that during the 1st day of the intoxication the output of urine was variable. In Experiments 4 and 6 a sharp reduction in the formation of urine occurred, while in the animal of Experiment 5 the output of urine was in excess of the average normal secretion. In all the animals, as the intoxication progressed, the formation of urine rapidly decreased. The amount of albumin in the urine was slight and is no indication of the severity of the kidney damage. Six of the animals of this group showed both glucose and acetone in the urine. The glucose generally appeared in the urine after the appearance of albumin. The appearance of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood. The elimination, however, of these bodies by the kidney does not show a

TABLE III.  
Group II. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		cc.	gm.	per cent	mg.	per cent		per cent			mm.			
4	15	1	312	Tr.	Tr.	14.7321	71	Heavy.	0.0167	7.48.0		40	Severe enteritis. Blood.	Frequent.	Bad.
		2	418	"	"	4.6371	28	Tr.	0.0217	3.7.9		32	No change.	Less frequent.	"
		3	121	"	"	3.7241	Tr.	"	0.0677	2.7.8		20	Less frequent.	Less frequent.	Very bad.
		4	0	0	0	0	0	0	0.0787	1.7.75		20	No change.	None.	Died in convulsions.
5	15	1	665	0	0	0	35	Tr.	0.0157	4.7.95		40	Severe enteritis. No blood.	Frequent.	Bad.
		2	31	Tr.	Tr.	Insufficient urine.	0	Insufficient urine.	0.0297	3.7.85		25	No change.	Occasional.	Very bad.
		4	0	0	0	0	0	0	0.0927	1.7.75		15	Less frequent. No blood.	None.	Died in air-hunger.
6	15	1	281	0.25	0	0	Tr.	Heavy.	0.0337	2.7.9		34	Severe enteritis. Blood.	Occasional.	Bad.
		2	31	0.75	Tr.	4.4784	"	Tr.	0.0447	1.7.55		15	Improved.	None.	"
		3	8	1.2	Insufficient urine.	Insufficient urine.	Insufficient urine.	Insufficient urine.	0.0817	1.7.5		12	No change.	"	Died in air-hunger.



parallel with the reduction in the alkali reserve. With the further depletion of the alkali reserve, and associated with the decreased elimination of phenolsulfonephthalein by the kidney, a decrease in the output of acetone occurs.

The elimination of phenolsulfonephthalein was reduced in all the experiments. The degree of reduction in the output of the dye varied in the different animals. There was no correlation between the quantitative output of phenolsulfonephthalein and the elimination of mercury by the kidney. In Experiment 4 a heavy amalgam of mercury was obtained from the urine of the animal secreted during the 1st day of the experiment, yet the phenolsulfonephthalein output remained high, 71 per cent. In Experiment 5, however, with only a trace of mercury in the urine, the output of phenolsulfonephthalein was reduced from 80 to 35 per cent.

The experiments show a relation between the phenolsulfonephthalein output and the reduction in the alkali reserve of the blood. The animals which showed a rapid depletion in the alkali reserve also showed a sharp decrease in the ability of the kidney to secrete the dye. Following the variation in the output of urine which was noted in the different animals early in the intoxication, as the intoxication progressed all the animals showed a rapid reduction in the formation of urine and became anuric. The anuria in this series of animals was not associated with a condition of shock as in the anuric animals of Group I. With the progressive decrease in the formation of urine the gastroenteritis lessened in severity. Associated with the reduction in the output of urine there occurred a rapid decrease in the elimination of phenolsulfonephthalein, a retention of blood urea, and the development of a severe acid intoxication. The animals dying in air-hunger showed the greatest depletion of the alkali reserve of the blood and the most marked decrease in the tension of alveolar air carbon dioxide. In Experiment 5, 2 hours before death the animal had a reserve alkali reading of 7.75 and a tension of alveolar air carbon dioxide of 15 mm. In Experiment 6 the alkali reserve was reduced to 7.5 and the tension of alveolar air carbon dioxide to 12 mm. None of the animals of the group survived longer than 7 days.

*Pathology of the Kidney.*—The kidneys from this group of animals were either removed immediately after death or the animals were

killed while in air-hunger and the organs secured for the pathological study. The kidneys are pale and swollen. On section the cortex bulges through the cut capsule. The cut surface appears pale and relatively bloodless. The microscopic examination has shown the glomeruli to be in a fair state of preservation. The capillaries are not engorged with blood. The endothelial nuclei are swollen and stain intensely. The epithelium of the tubules, except that of the junctional tubules, has shown a remarkable degree of swelling and early necrosis. The swollen cells in many tubules have not only completely occluded the lumen of the tubule, but give to the tubule an increased transverse diameter. The nuclei are large and hypochromatic. In other tubules the nuclei have disappeared, the cells having undergone complete necrosis. (Fig. 2.)

### *Group III.*

The third group of experiments consists of the animals which received mercuric chloride and, after recovering from the mercury enteritis, developed an acid intoxication and later made a complete recovery. In this group of animals which did not develop the severe gastroenteritis characteristic of the early stage of the intoxication in the two previous groups, the output of urine was not greatly reduced (Table IV). Albumin was present in the urine of all the animals with two exceptions. The amount did not exceed 1 gm. per liter. With the appearance of albumin in the urine the phenolsulfonephthalein output was decreased, a reduction occurred in the alkali reserve of the blood and in the tension of alveolar air carbon dioxide, and acetone bodies appeared in the urine. Usually during the 2nd day these changes in the urine and blood had reached their maximum and from this point in the experiments the animals showed a return to the normal both in regard to the functional capacity of the kidney, and the restoration of the normal acid-base equilibrium of the blood. Since this series of animals went to the stage of recovery, a study of the experiments permits an investigation of the relation between the elimination of mercury by the kidney and the development of the kidney injury. In Experiment 9, Table IV, on the 1st day of the intoxication a heavy precipitate of mercury was obtained from the urine. The amount of urine formed showed an increase over the

TABLE IV.  
Group III. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
7	15	1	621	0	0	0	85	Heavy Tr.	0.0127.3	8.0	38	38	Enteritis. No blood.	None.	Good.
		2	297	0	0	5.4114	85	Tr.	0.0127.4	7.95	34	34	" improved.	"	Improved.
		4	343	0	0	5.1315	84	"	0.0127.45	8.05	40	40	Normal.	"	Appears normal.
		10	497	0	0	11.6625	87	0	0.0127.55	8.1	45	45	"	"	Recovery.
8	15	1	655	0.5	0	7.5321	78	Heavy Tr.	0.0197.4	7.95	38	38	Severe enteritis. Blood.	Occasional.	Bad.
		2	283	1.0	2.15	12.4210	66	Amount decreased.	0.0197.4	7.95	38	38	Improved.	None.	Improved.
		4	450	Tr.	0.2	18.3241	80	0	0.0157.5	8.1	45	45	Normal.	"	"
		8	670	0	0	17.6714	82	0	0.0157.5	8.1	45	45	"	"	Recovery.
9	15	1	750	0	0	2.5657	85	Heavy Tr.	0.0157.45	8.0	42	42	Severe enteritis. No blood.	Occasional.	Good.
		2	580	Tr.	0	6.1885	75	"	0.0267.4	7.9	35	35	Enteritis improved.	"	Improved.
		3	623	"	Tr.	6.2431	83	Amount greatly increased.	0.0227.5	8.0	40	40	Improved.	None.	"
		6	220	"	"	11.8957	86	0	0.0157.45	8.05	40	40	"	"	"
		10	481	0	0	4.7382	85	0	0.0137.45	8.05	40	40	Normal.	"	Normal.
		12	531	0	0	0	85	0	0.0137.45	8.05	40	40	"	"	Recovery.

normal, the elimination of phenolsulfonephthalein showed practically no reduction, 86 to 85 per cent, and the reserve alkali of the blood remained unchanged. On the 2nd day of the experiment the elimination of mercury was still heavy, while the formation of urine remained nearly normal. The phenolsulfonephthalein output was reduced only 10 per cent. The alkali reserve of the blood was reduced from 8 to 7.9. On the 3rd day of the experiment the elimination of mercury was greatly increased. There was, however, an increased formation of urine, 623 cc., the output of phenolsulfonephthalein had returned to 83 per cent, the normal output being 86 per cent, and the alkali reserve of the blood had returned to the normal reading. Experiments 7 and 8 also illustrate the lack of relation between the elimination of mercury by the kidney and the decrease in the functional capacity of the organ.

#### *Group IV.*

The animals comprising this group developed a moderately severe mercury enteritis. During the enteritis, or following its subsidence, the animals developed an acid intoxication which varied in degree and duration. In this respect this group of animals resembles the experiments of Group III. The animals of Group III, however, gradually returned to the normal and made a complete recovery, while the animals of Group IV, after a period during which there was an attempt to restore the normal acid-base equilibrium, became severely intoxicated and showed a more marked depletion of the alkali reserve than was obtained in the initial acid intoxication. The formation of urine was rapidly reduced. The animals were anuric from 1 to 6 days before death.

A study of Table V, which gives in detail the results obtained in one of the experiments of this group, shows that the secretion of urine was rapidly reduced. Associated with the reduction in the output of urine the animals became albuminuric, and later both glucose and acetone appeared in the urine. The amount of albumin was small, not over 0.9 gm. per liter. The glycosuria rapidly disappeared. The output of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood but did not show quantitatively



a parallel with the decrease in the alkali reserve. As the acid intoxication progressed the elimination of acetone bodies was diminished. The decrease in the elimination of phenolsulfonephthalein is not proportionate to the decreased formation of urine. In this group of animals, as in the other groups, there is more nearly a correlation between the degree of acid intoxication and the elimination of the dye than there is between the output of urine and its elimination. Associated with the reduction in the output of phenolsulfonephthalein a retention of blood urea occurs. The stage of improvement in this group of animals was characterized by an increased formation of urine, a decrease and final disappearance of albumin from the urine, an increase in the output of acetone bodies and phenolsulfonephthalein, a decrease in the percentage of blood urea, and a return towards the normal acid-base equilibrium of the blood. None of these conditions reached the point of normality. From the 6th to the 10th day in Experiment 10 the urine was free from albumin, the phenolsulfonephthalein elimination was 48 to 51 per cent, and the reserve alkali 7.9 to 7.95. On the 11th day the terminal acid intoxication commenced. The following day only 81 cc. of urine were formed. The urine was free from albumin. The phenolsulfonephthalein elimination had been reduced from 51 to 13 per cent and the alkali reserve was reduced from 7.95 to 7.75. The animal was anuric the following day and died in air-hunger.

A study of the elimination of mercury by the animals of this group is of special interest. During the entire course of Experiment 10, Table V, only a trace of mercury was found in the urine. After the 4th day of the intoxication the urine was free from mercury. As will be seen by referring to the table, the kidney damage which was associated with the death of the animal did not occur until the 11th day of the experiment, 7 days after the urine had been free from mercury.

*Pathology of the Kidney.*—The kidneys of this group of animals have been pale and relatively bloodless. Extending from the corticomedullary junction into the cortex are streaks of fatty degeneration. The histological examination has shown the glomeruli shrunken and not infrequently surrounded by an exudate of serum, fibrin, and red cells. The endothelium of the glomerular capillaries has shown an

advanced degeneration. The nuclei have shown fragmentation. The loops of capillaries are fused together. The epithelium of the tubules has shown an advanced necrosis. The structure of the cells is completely lost. The tubules are outlined by a structureless mass of necrotic material. The tubules have frequently been separated by an exudate containing red cells and fibrin. (Fig. 3.)

#### SUMMARY.

A study of the experiments comprising the first group of animals permits the deduction that these animals succumb to the acute poisoning as a result of the shock which the poison induces through its corrosive action in the stomach and intestine. The animals die before the mercury, acting as such during its elimination by the kidney, can induce an acute nephropathy and before the mercury, by inducing an acid intoxication, can lead to an acute kidney injury.

The remaining animals of the series, Groups II, III, and IV, have withstood the corrosive action of the poison. These animals have shown the same type of delayed intoxication from the poison. The intoxication, however, has varied in time of appearance, duration, and severity.

The animals classified as Group II have developed during the stage of improvement from the gastroenteritis a rapid and severe type of acid intoxication, have become rapidly anuric, and have died either in a state of air-hunger or in convulsions.

The animals of Group III, either during or after their recovery from the gastroenteritis, have developed a mild grade of acid intoxication. During the following days of the experiments the animals succeeded in reestablishing their normal acid-base equilibrium. All the animals of this group recovered.

The animals of Group IV have shown a recovery from the mercury enteritis. Following a period during which there was an attempt on the part of the animals to return to normal, as indicated by an increase in the alkali reserve of the blood and by an increased output of phenol-sulfonephthalein and urine, the members of the group developed a delayed acid intoxication, and, like the animals of Group II, became anuric.

The animals of all groups which have died from the delayed intoxication caused by the mercury have shown a severe type of kidney injury which has been characterized by an acute swelling and necrosis of the renal epithelium. All these animals have either gradually or acutely developed a severe type of acid intoxication. There has been a definite association between the development of an acid intoxication and the delayed kidney injury, and, furthermore, the animals which have shown the greatest swelling and necrosis of the renal epithelium have also shown the severest type of intoxication.

#### CONCLUSIONS.

1. In the acute mercuric chloride intoxications which have been induced in dogs death has been due either to the shock associated with the severe mercury enteritis or to a delayed kidney injury.

2. The injury to the kidney has been constantly associated with the development of an acid intoxication.

3. The delayed kidney injury is not due to the action of the mercury as such during its elimination by this organ.

4. The manner in which mercuric chloride induces an acid intoxication is at present under investigation. The participation of the liver in the intoxication will be considered in this connection.

#### EXPLANATION OF PLATES.

##### PLATE 14.

FIG. 1. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 3, Table II. The glomerulus, *a*, appears normal except for the deeply staining endothelial nuclei. The epithelium of the convoluted tubules, *b*, shows the presence of albuminous granules with little increase in the size of the epithelial cells. The nuclei stain well. The convoluted tubule epithelium at *c* shows an early swelling of the cells. The animal died in collapse associated with the corrosive action of mercuric chloride.

##### PLATE 15.

FIG. 2. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of Experiment 6, Table III. It shows at *a* the histologically well preserved glomerular capillaries. The endothelial nuclei are swollen and stain intensely. At *b* is shown the severe swelling of the convoluted tubule epithelium



which has occluded the lumen of the tubule and increased the size of the tubule. The nuclei of these cells are large and hypochromatic. At *c* are shown tubules that have become completely necrotic. The junctional tubules, *d*, do not show the severe swelling and necrosis which has developed in the more highly specialized tubular epithelium. The animal became anuric from the mercuric chloride intoxication and died in air-hunger.

PLATE 16.

FIG. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 10, Table V. It shows at *a* the glomerulus with fused capillary loops containing endothelial nuclei which have undergone fragmentation. The convoluted tubules at *b* have become completely necrotic. The outline of the tubule is formed of a structureless, necrotic mass. At *c* an exudate is shown separating the tubules, which consists of serum, fibrin, and red blood cells. The figure illustrates the changes in the kidney of the animals of Group IV, Table V, which recover from the mercury enteritis and later succumb from the delayed intoxication which is associated with the development of an acid intoxication.

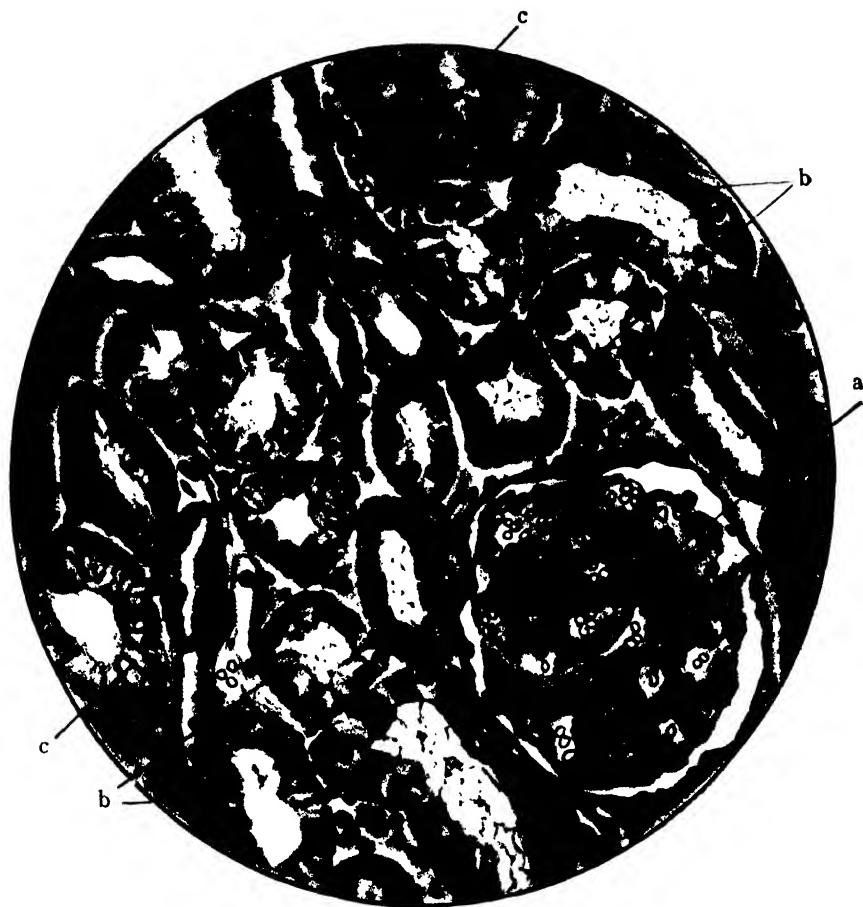


FIG. 1.



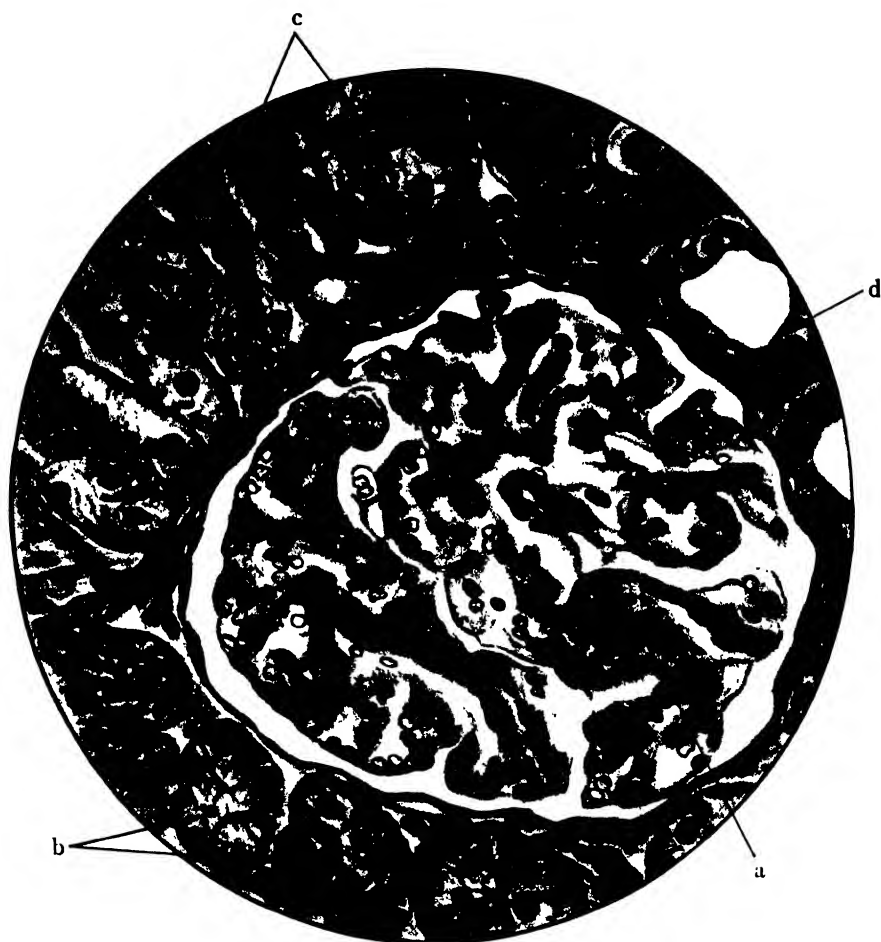


FIG. 2.

(MacNider: Mercuric chloride intoxications.)



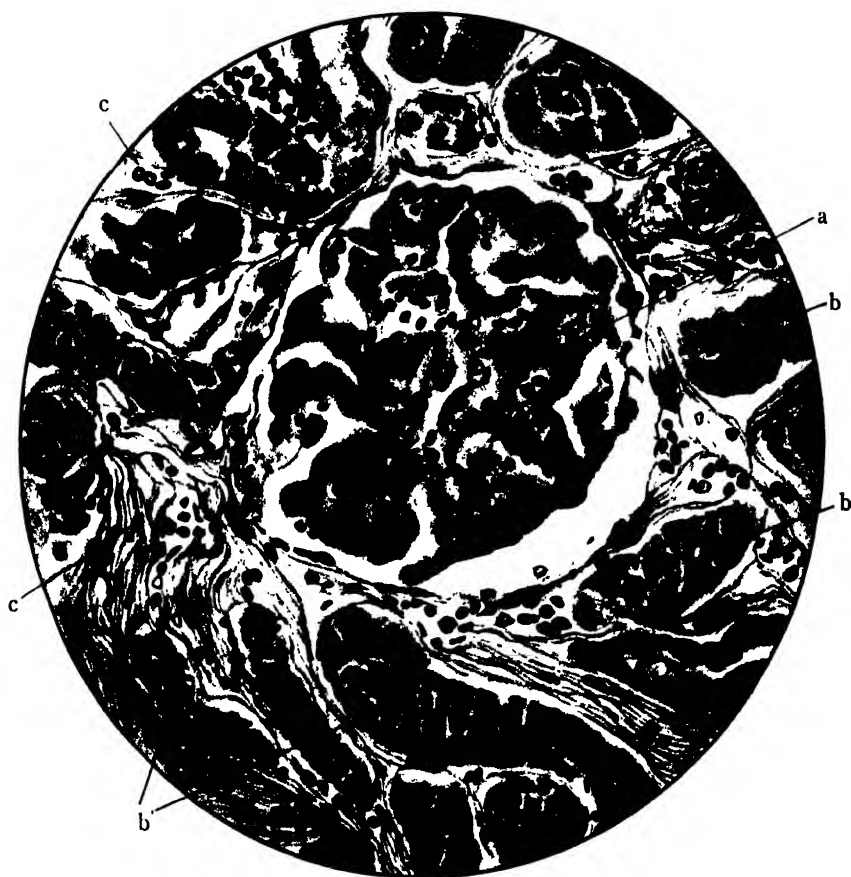


FIG. 3.

(MacNider: Mercuric chloride intoxications.)



production. Each strain produces a toxin which, on animal inoculation, gives rise to lesions comparable in every respect to those produced by the toxins previously reported on,<sup>1</sup> and each toxin was neutralized by an immune (antitoxic) serum produced with one of the former toxins. The toxins obtained from the several individual strains varied in potency, the lethal dose ranging from 0.3 to 3 cc.

Experiments have been made to determine the influence of fresh muscle and glucose on toxin production and the relation of acidity to toxicity in the filtrates. It has been found that the addition of fresh muscle to the medium increases the potency of the toxin five-fold. Autoclaved muscle is without effect. Beef infusion broth containing 0.2 to 1 per cent glucose gives a more potent product than sugar-free broth, while when higher percentages are employed the toxin production is lowered. There is no direct relation between acidity and toxicity, the most acid products manifesting little or no toxic action. In every medium used for culture the potency of the filtrates rapidly diminished after 24 hours' incubation, while the acidity increased or remained constant. The exception to this rule has been pointed out.

The most active toxin is obtained by growing a virulent strain of the bacilli in a 0.2 or 0.3 per cent glucose broth to which fragments of fresh muscle have been added, and collecting the filtrate after from 18 to 24 hours' incubation.

A review of the literature on the pathogenic effects and toxic products of *Bacillus welchii* and on the results of immunization of animals with the bacilli or toxic products does not indicate that the exotoxic nature of *Bacillus welchii* had been previously determined or an antitoxic serum in the true sense produced.

The antitoxin for *Bacillus welchii* toxin can apparently be prepared from a single strain of the organism which yields under the conditions described a high titer of toxin, and this antitoxin can be employed to combat infection with or prevent infection by any strain whatever of the bacillus.





## A STUDY OF MITOCHONDRIA IN EXPERIMENTAL POLIOMYELITIS.

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### INTRODUCTION.

Although many observations have been made on mitochondria in normal tissues, both adult and embryonic, the study of these structures in pathological material has been relatively limited and the results in some cases have been conflicting. The information regarding this type of cell granule has been summarized by Cowdry,<sup>1</sup> but in view of the fact that several observers have recorded changes in mitochondria, often occurring quite early in certain lesions,<sup>2-13</sup> it seemed possible that some such results might be obtained in the case of poliomyelitis, which would throw light on the pathology of that condition.

Spinal ganglia were employed for the study, because they show typical lesions in monkeys dying of experimental poliomyelitis, and because, of the structures showing these lesions, they could be most

<sup>1</sup> Cowdry, E. V., *Am. J. Anat.*, 1916, xix, 423.

<sup>2</sup> Barrett, J. O., *Quart. J. Micr. Sc.*, 1913, lviii, 214.

<sup>3</sup> Beckton, H., *Arch. Middlesex Hosp.*, 1909, xv, 182.

<sup>4</sup> Beckton, H., and Russ, S., *Arch. Middlesex Hosp.*, 1911, xxiii, 59.

<sup>5</sup> Bensley, R. R., *Tr. Chicago Path. Soc.*, 1909-12, viii, 78.

<sup>6</sup> Ciaccio, C., and Scaglione, S., *Beitr. path. Anat. u. allg. Path.*, 1913, lv, 131.

<sup>7</sup> Goetsch, E., *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 29.

<sup>8</sup> Homans, J., *J. Med. Research*, 1915-16, xxxiii, 1.

<sup>9</sup> Regaud, C., and Favre, M., *Compt. rend. Soc. biol.*, 1911, lxviii, 658.

<sup>10</sup> Regaud and Favre, *Compt. rend. Soc. biol.*, 1912, lxix, 328.

<sup>11</sup> Romes, B., *Anat. Anz.*, 1913-14, xlv, 1.

<sup>12</sup> Scott, W. J. M., *Am. J. Anat.*, 1916, xx, 237.

<sup>13</sup> Strongman, B. T., *Anat. Rec.*, 1917, xii, 167.

successfully obtained and fixed. Cowdry<sup>14</sup> has given a full account of the mitochondria occurring in normal ganglion cells of vertebrates.

#### EXPERIMENTAL.

In a few instances injection fixation was employed. Here the method described by Cowdry<sup>1</sup> was used with the following variations. The formalin bichromate mixture was more successful when used in half rather than in full strength. 2 to 3 feet of gravity pressure of injection fluid were found to give less edema and distortion of the tissues than the higher pressure (4 to 6 feet), so that while the saline infusion was given at the higher pressure for the first 5 to 10 minutes to insure complete washing out of clots, the lower pressure was maintained throughout the remainder of the period. The femoral vein of one side was cut, rather than the vena cava, as the latter gave too rapid an outflow of fluid. The cardiac, mesenteric, and opposite iliac arteries were clamped. The injection of fixative was continued for 1 hour and the best results were obtained when the animal was kept lying on the board back down, for  $\frac{1}{2}$  hour longer before autopsy.<sup>15</sup> Injection was less successful in poliomyelitic monkeys and in the lumbar region of operated rabbits than in normal animals, possibly owing to vascular lesions in the former.

The ganglia, however, were quite as well fixed by simple immersion in fixing fluid. The animals were usually chloroformed and the ganglia taken at once. In a few cases the animals died and were autopsied within 2 hours of death. The specimens were placed in formalin bichromate mixture (3 per cent potassium bichromate 4 parts, neutral formalin 1 part, and water 5 parts) for 4 days and then transferred to half strength ( $1\frac{1}{2}$  per cent) potassium bichromate for 5 days. This procedure was found to give better results than the solution ordinarily used. Full strength solutions and the acetic-osmic-bichromate mixture<sup>16</sup> (2.5 per cent bichromate 8 cc., 2 per cent osmic acid 2 cc., and glacial acetic acid 1 drop) in full strength or diluted to half strength were apt to give good fixation only in the

<sup>14</sup> Cowdry, *Am. J. Anat.*, 1914-15, xvii, 1.

<sup>15</sup> Schirokogoroff, J. J., *Anat. Anz.*, 1913, xliii, 522.

<sup>16</sup> Bensley, *Am. J. Anat.*, 1911-12, xii, 297.

case of superficial cells. The osmic acid mixture also darkened the whole section to such an extent that it dimmed the contrast between mitochondria and small Nissl bodies.

At first the use of chloroform in embedding was tried, but in many cases the mitochondria disappeared under this treatment. In one case the tissue was first embedded by the xylol method and sections were cut, then reembedded by the chloroform method.<sup>17</sup> The former sections showed mitochondria, but none was present in the latter. This may have been due to rehandling, however. Embedding was done as described by Cowdry,<sup>14</sup> except that absolute alcohol-xylol for 1 hour, xylol 1 hour, paraffin 3 hours, was found to be sufficient and less liable to destroy the mitochondria. Both the acid fuchsin-methyl green, and the iron-alum-hematoxylin (Regaud and Favre<sup>9</sup>) methods were used for staining.

*Rabbits.*—In order to compare cell changes in another form of paralysis a series of rabbits was used, in some of which ischemic paralysis of the hind legs was produced by the Stenson operation (Fredericq,<sup>18</sup> Ehrlich and Brieger<sup>19</sup>). The animals were etherized. An area about 3 inches wide, extending from ensiform process to symphysis pubis, was shaved and cleaned with alcohol. Aseptic technique was employed. An incision was made in the midline, and the intestines were covered with cloths wet with warm saline. The abdominal aorta was exposed and a soft bulldog clamp placed on it about  $\frac{1}{2}$  inch below the renal arteries, the effectiveness of the clamp being tested by palpation of the vessel below it. The intestines were replaced and the animal was kept under light ether anesthesia, the clamp being left in place for  $\frac{1}{2}$  or  $\frac{3}{4}$  hour. The longer period was found to give certain results while the former failed in some cases to give paralysis. Care was taken to keep the animal warm during this period. At the end of the period the clamp was removed, the abdominal wall sewed with silk, and the animal allowed to recover.

In almost every instance, flaccid paralysis of the hind legs was evident as soon as the animal recovered from the ether. In two rabbits

<sup>17</sup> Mallory, F. B., and Wright, J. H., *Pathological technique*, Philadelphia and London, 6th edition, 1915, 284.

<sup>18</sup> Fredericq, L., *Arch. biol.*, 1890, x, 131.

<sup>19</sup> Ehrlich and Brieger, *Z. klin. Med.*, 1884, vii, Supplement, 155.

in which the shorter compressions were used, the hind legs were spastic, with convulsive twitchings, which gradually disappeared, the animal recovering the full use of the legs.

The animals were chloroformed at various periods after the ligation and fixed by the injection method. The lumbar region of the cord did not always take the fixative so well as did the cervical region, or the lumbar region of normal animals, and in those animals killed 12 or more hours after the clamping, the cords remained extremely soft, and were uncolored by the chromate and very difficult to handle.

*Material.*—A series of ganglia was obtained from fourteen monkeys with experimental poliomyelitis.<sup>20</sup> Six were either in the preparalytic stage, without definite lesions in the ganglia, though having shown such symptoms as irritability, etc., or if they showed paralysis, the particular ganglia used failed to show typical lesions. The remaining ten, taken from the 1st to the 7th day after the onset of paralysis of some muscle group had been noted, all showed typical lesions of the cord and ganglia, including those used for mitochondria, as shown by examination in gross or of sections stained by hematoxylin and eosin.

Five monkeys were used as controls. Two of these showed lesions of tuberculosis at autopsy. One was an apparently normal monkey which died while being etherized, and the viscera showed no gross abnormalities. The other two monkeys had received poliomyelitis virus intranasally but showed no symptoms either during life or post mortem.

*Results.*—The ganglia from the five control monkeys and those from the six poliomyelitic monkeys presenting no lesions in the ganglia used showed mitochondria similar to those described by Cowdry in the normal animal. Great variations were found in the number of mitochondria and the intensity with which they took the stain, and several showed many cells in the chromatophilic state.<sup>21</sup> In one, considerable postmortem degeneration had occurred.<sup>22</sup> One showed a large amount of typical lipid. All, however, were cells similar to those described by Cowdry as normal cells. This was true also

<sup>20</sup> Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

<sup>21</sup> Cowdry, Contributions to embryology, *Carnegie Institution of Washington, Publication No. 224*, 1916, Contribution No. xi.

<sup>22</sup> Ciaccio, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 721.

of the ganglion cells from the normal rabbits and those from the cervical region of the operated rabbits. In these rabbit sections, no chromatophil cells were found.

In the tissues from the poliomyelitic animals also, many cells were normal in appearance. In many, moreover, the mitochondria appeared to be even more clearly shown than in normal cells. This appearance may have been due to disappearance of Nissl substance, which was reduced in these cells. In normal cells it was often hard to differentiate between mitochondria and small Nissl bodies as the granules were of nearly the same size and in some preparations tended to take the fuchsin stain. Many cells contained much particulate lipid and many were in the chromatophilic state, but in these particulars they did not seem to exceed normal limits.

In the cells which showed marked neurophagocytosis, mitochondria-like threads could often be seen, even though only a small remnant of protoplasm remained. They appeared as minute reddish threads or dots, or larger masses, even to fairly large rods, lying in the usual bluish background and in spaces between the invading cells. They did not have the globular shape characteristic of lipid, and they seemed to have some of the chemical reactions of the mitochondria, for they were not found in slides in which the mitochondria had been lost through poor fixation. In cells in which the destruction had been less complete, typical mitochondria were found to persist in an apparently normal ratio to cell substance.

A similar, though less marked persistence of mitochondria was also noted in the lumbar ganglia of the rabbits. 1 hour after the production of the anemia, a few darkly staining cells were found, showing a few reddish threads against a dark purplish background. At 7 hours almost all the cells had this chromatophilic tendency, a few still showing the reddish threads, but in the majority there was only a shrunken, irregularly stained protoplasm. At 12 hours, the position of a few remaining cells was indicated by dark, indefinite spots.

#### DISCUSSION.

If these red-staining threads occurring in the invaded cells in poliomyelitis are mitochondria, the mitochondria in this condition

at least outlast any other cell structure now recognized. This is remarkable in view of their usual tendency to disappear under slight changes, such as acidity or temperature. Since the stain is not absolutely specific, and we lack means at the present time of differentiating mitochondria with certainty from other lipoidal structures, it may be that these threads, or rods, are merely part of a coagulum of some different nature. Yet, since typical mitochondria persist after the disappearance of typical Nissl substance, and various gradations can be traced up to the stage of almost total replacement of the original ganglion cell, it would seem safe to call these mitochondria under the present use of the term.

It would be desirable to have some means of quantitative estimation of the mitochondria for these purposes, but the one so far described (Thurlow<sup>23</sup>) does not prove applicable to the rod-like forms.

#### CONCLUSION.

Typical mitochondria can be found in the spinal ganglion cells of monkeys with experimental poliomyelitis, even when typical Nissl substance has disappeared, and mitochondria-like structures are found in the remaining protoplasm in the latest stage of neurophagocytosis.

<sup>23</sup> Thurlow, M., Contributions to embryology, *Carnegie Institution of Washington, Publication No. 226*, 1917, Contribution No. xvi.

## THE SOLVENT ACTION OF ANTISEPTICS ON NECROTIC TISSUE.

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PLATE 5.

(Received for publication, October 1, 1917.)

The recent interest in the chemical sterilization of wounds has led to the introduction of numerous new antiseptics, each of which has in turn been advocated because of some advantage, real or apparent. For many of these compounds, claims have been made which are not always confirmed by carefully controlled experiments. Carrel and Dehelly<sup>1</sup> emphasize that, for the removal of the necrotic tissue that remains after mechanical cleansing, Dakin's hypochlorite solution is the antiseptic of choice because of its solvent action on devitalized tissue, and Dakin and Dunham<sup>2</sup> also recognize the value of the hypochlorite solution for this purpose.

Dakin's solution was shown by Fiessinger and his coworkers<sup>3</sup> to have a disintegrating action on pus cells. Rous and Jones<sup>4</sup> have shown that intact leukocytes may protect virulent bacteria which they have phagocyted from the action of antiseptics, and that subsequently these bacteria may proliferate under suitable conditions. Dakin's solution, by its solvent action on these leukocytes, minimizes the danger of reinfection of the wound from this source. Because of this action on necrotic tissue, pus, and serum clot, Carrel and Dehelly recommend Dakin's hypochlorite solution for the sterili-

<sup>1</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 150, 192.

<sup>2</sup> Dakin, H. D., and Dunham, E. K., *A handbook on antiseptics*, New York, 1917, 14.

<sup>3</sup> Fiessinger, N., Moiroud, P., Guillaumin, C. O., and Vienne, G., *Ann. med.*, 1916, iii, 133.

<sup>4</sup> Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.



zation of infected wounds.<sup>5</sup> Bashford<sup>6</sup> has demonstrated the ability of Dakin's solution in high dilution to erode the tissues of the tadpole's abdomen. He showed also that this occurred only after the circulation to the part had been interrupted for some time, due to the death of the organism.

As this erosive action of Dakin's solution is an important factor, the following experiments were planned to compare its solvent action with that of certain other chlorinated antiseptics. Fiessinger and his coworkers<sup>3</sup> concluded that the essential factor in the solvent action of the hypochlorites is their alkalinity. Our experiments were therefore designed to determine the importance of three factors: the alkalinity, the nature of the chlorinated antiseptic employed, and the chlorine concentration of the latter.

#### *Method.*

The solvent action of the various substances employed was tested by adding 50 cc. of each solution to 5 cc. of an emulsion of macerated liver tissue in a 100 cc. bottle. The mixture was thoroughly shaken every half hour for 2 hours. A 15 cc. portion was then removed to a centrifuge tube and in each case centrifuged at the same high speed for 5 minutes. The volume in cubic centimeters of the sediment thrown down was measured. The solvent action was shown by diminution of the amount of sediment compared with that obtained from inert solutions such as water or normal saline solution.

The liver emulsion was prepared in Experiment 1 from rabbit liver, in the other experiments from cat liver. In Experiments 1, 2, 3, and 4 the liver was purposely infected by handling, placed in the incubator at 37°C. until thoroughly necrotic, cut into small pieces, suspended in saline solution, shaken in a bottle with broken glass to emulsify it, and strained through a single layer of gauze. In Experiment 5 cat liver was similarly emulsified and used after 12 hours' preservation in the ice box. The solutions employed were prepared as follows:

<sup>5</sup> Carrel, A., and Dehelly, G., *The treatment of infected wounds*, New York, 1917, 109.

<sup>6</sup> Bashford, E. F., *Lancet*, 1917, cxcii, 595.

*Control Solutions.*—Neutral: water and normal saline solution. Weakly alkaline: a solution of sodium carbonate, 1 gm., and sodium bicarbonate, 17 gm. per liter of water; this solution has approximately the alkalinity of a properly prepared Dakin's solution. Strongly alkaline: 0.1 N sodium hydroxide.

*Chloramine-T Solutions.*—Chloramine-T solutions were prepared by dissolving the required amount of chlorazene<sup>7</sup> in the appropriate control solution to obtain neutral, weakly alkaline, or strongly alkaline chloramine solutions.

*Hypochlorite Solutions.*—Weakly alkaline: ordinary Dakin's solution prepared either from bleaching powder or by the action of liquid chlorine on sodium carbonate solution, in either case with careful control of the degree of alkalinity as well as of the hypochlorite content. Neutral: chlorine gas passed through sodium carbonate solution, 28 gm. per liter, until the solution just ceased to give a flash of pink upon the addition of alcoholic solution of phenolphthalein, 1 per cent; the hypochlorite content was determined by titration, and the solution diluted with water to the desired strength. This solution was always prepared immediately before use, as a considerable proportion of the total hypochlorite is present as hypochlorous acid, and the decomposition of the solution is very rapid (Cullen and Austin<sup>8</sup>). Strongly alkaline: A double strength neutral solution of hypochlorite was prepared as just described and immediately added to an equal volume of 0.2 N sodium hydroxide solution.

*Chlorinated Oils.*—Paraffin oil and eucalyptol were mixed in equal parts, and the same oils chlorinated according to Dakin's method.<sup>9</sup>

*Dichloramine-T.*—A 15 per cent solution was made in chlorinated eucalyptol and then mixed with an equal volume of chlorinated paraffin oil. In the experiments in which the oils were used (Experiment 1, Tubes 5, 6, and 7; Experiment 2, Tubes 4, 5, and 6; Experiment 3, Tube 9), except in Experiment 3, Tube 10, 5 cc. of liver emulsion were added to 50 cc. of water or of one of the control solutions, and 15 cc. of the oil used were superimposed upon this mixture; the mixture was well shaken every half hour with a rotary motion.

<sup>7</sup> Prepared by the Abbott Laboratories, New York.

<sup>8</sup> Personal communication.

<sup>9</sup> Dakin, H. D., *Brit. Med. J.*, 1915, ii, 318.

At the end of 2 hours, after again being shaken, the oil was allowed to separate from the aqueous suspension and 15 cc. portions were immediately removed from the latter for centrifuging. In Tube 10 of Experiment 3, the 5 cc. of liver emulsion were introduced into 50 cc. of the oil without water and shaken continuously for 2 hours.

The sodium hypochlorite equivalent of the hypochlorite and other chloramine solutions was determined by the addition of potassium iodide solution and glacial acetic acid to 10 cc. samples and titration of the iodine liberated with 0.1 N sodium thiosulfate.

### RESULTS.

In Experiment 1, Table I, Solutions 8 and 9 being taken as controls, no solvent effect was noted after the action of chloramine-T solution or of the chlorinated oils, with or without dichloramine-T. Dakin's solution, on the other hand, had a marked solvent action, which was apparent upon inspection of the bottles, even in the first 15 minutes. This was accompanied by pronounced bleaching of the emulsion. A still more rapid and marked action, similar in character, was obtained from the strongly alkaline hypochlorite. Figs. 1 and 2 show the results of this experiment at the end of 2 hours. The reaction of the

TABLE I.  
*Experiment 1.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral chloramine-T, 0.5 per cent, equivalent to sodium hypochlorite, 0.12 per cent. ....	0.30
2	Neutral chloramine-T, 0.2 per cent, equivalent to sodium hypochlorite, 0.5 per cent. ....	0.25
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's solution)) . . . . .	0.05
4	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent). . . . .	0.02
5	Water + paraffin oil and eucalyptol.* . . . .	0.27
6	" + chlorinated paraffin oil and eucalyptol.* . . . .	0.28
7	" + " " " " " + dichloramine-T, 7.5 per cent. . . . .	0.27
8	Salt solution . . . . .	0.28
9	Water . . . . .	0.27

\* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil.

solutions used, however, varied, as well as the nature of the antiseptic substance. Experiment 2 was therefore performed with solutions of approximately the same reaction (Table II).

The results of this experiment confirm those observed in Experiment 1. Chloramine-T and dichloramine-T were without solvent action, whereas Dakin's hypochlorite gave marked solution. In Experiment 3, Table III, the effect of diminishing the concentration of the antiseptic in a solution of the same reaction was tested. Both weakly alkaline and strongly alkaline solutions were employed.

TABLE II.  
*Experiment 2.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Weakly alkaline carbonate-bicarbonate solution.....	1.27
2	“ “ “ “ + chloramine-T, 2 per cent.....	1.24
3	Weakly alkaline hypochlorite, stock Dakin's solution (sodium hypochlorite, 0.5 per cent) .....	0.08
4	Weakly alkaline carbonate-bicarbonate solution + paraffin oil and eucalyptol.*.....	1.23
5	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol.*.....	1.24
6	Weakly alkaline carbonate-bicarbonate solution + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.....	1.32

\* 5 cc. of liver emulsion in 50 cc. of carbonate-bicarbonate solution, overlaid with 15 cc. of oil.

This experiment showed a loss of the solvent action in weakly alkaline hypochlorite solutions, occurring suddenly between 0.2 and 0.3 per cent sodium hypochlorite concentration. In the strongly alkaline solutions the solvent action was marked, even at the lowest hypochlorite concentration employed. This experiment, taken in conjunction with the well known rapid drop in the sodium hypochlorite titer of Dakin's solution in contact with tissues, indicates that any solvent action resulting from its application clinically may be expected to occur in the first few minutes and emphasizes the importance of frequent flushing of wounds with the solution.

In order to distinguish between the effects of alkalinity and of hypochlorite concentration, Experiment 4 was performed (Table IV). The neutral hypochlorite solutions were prepared as described above. For the neutral control, saline solution was employed. The weakly alkaline hypochlorite solutions in the column headed "Weakly alkaline due to hypochlorite" were prepared as already described, except that the chlorine gas was passed into sodium hydroxide solution instead of into sodium carbonate, thus producing a solution of which

TABLE III.

*Experiment 3.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Water.....	0.25
2	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.22
3	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)....	0.01
4	" " " " " 0.4 " " ....	0.01
5	" " " " " 0.3 " " ....	0.02
6	" " " " " 0.2 " " ....	0.22
7	Strongly " " " " " 0.5 " " ....	Tr.
8	" " " " " 0.2 " " ....	0.08
9	Water + chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent.*.....	0.25
10	Chlorinated paraffin oil and eucalyptol + dichloramine-T, 7.5 per cent, without water.†.....	0.22

\* 5 cc. of liver emulsion in 50 cc. of water, overlaid with 15 cc. of oil. Shaker continuously for 2 hours.

† 5 cc. of liver emulsion in 50 cc. of oil. Shaken continuously for 2 hours.

the alkalinity was due almost entirely to sodium hypochlorite, and which was without buffer substances. The hypochlorite solution in the column headed "Weakly alkaline due to carbonate-bicarbonate" and the strongly alkaline solution were prepared exactly as described above. Table IV shows that in the control solutions solvent action occurred only in the strongly alkaline solution. A somewhat more marked solvent action was obtained when hypochlorite, even in the low concentration of 0.1 per cent, was added to the alkali. No solvent action was obtained in the weakly alkaline control, as compared

with the neutral control of normal saline solution. When hypochlorite was added to the weakly alkaline carbonate-bicarbonate solution, or when a weakly alkaline solution of sodium hypochlorite without carbonates was prepared, no solvent action was present at a concentration of 0.1 per cent, but it was marked at a concentration of 0.2 per cent. The change in solvent action resulting from small variations in hypochlorite concentration at about 0.2 per cent was striking, confirming the results of Experiment 3. The absence of solvent action of Dakin's solution below a hypochlorite concentration of 1:500 contrasts sharply with the marked bactericidal action of the same solution in serum to 1:1,500, and in water to 1:500,000 on *Staphylococcus aureus*.<sup>10</sup> In neutral solution at a hypochlorite concentration of 0.2 per cent, solvent action was very slight. At 0.3 per cent it was moderate, and at 0.5 per cent marked.

TABLE IV.

*Experiment 4.*

Tube No.	Hypochlorite concentration of solutions.	Reaction of solutions.			
		Neutral.	Weakly alkaline.		Strongly alkaline.
			Due to carbonate-bicarbonate.	Due to hypochlorite.	
		Sediment.	Sediment.	Sediment.	Sediment.
	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
1	0.5	0.01	0.01	0.01	Tr.
2	0.3	0.04	0.01	0.01	"
3	0.2	0.10	0.01	0.01	"
4	0.1	0.14	0.13	0.12	"
5	Control.	0.12	0.12		0.04

Experiment 5 serves as a final control of the solvent action of alkali alone, of chloramine-T added to neutral, weakly alkaline, and strongly alkaline solutions, and of hypochlorite solutions of the three grades of alkalinity. It is clear that chloramine-T, even in a 2 per cent solution, has no solvent action except that due to the alkalinity of the solution in which it is dissolved, and that therefore it is without this

<sup>10</sup> Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160.

action in the grade of alkalinity permissible for clinical use. On the other hand, 0.5 per cent neutral sodium hypochlorite-hypochlorous acid solution (Tube 7) has a marked solvent effect, which must be attributed to the action of the chlorine unaided by alkali. Fig. 3 shows the results of Experiment 5 (Table V) at the end of 2 hours.

TABLE V.  
*Experiment 5.*

Tube No.	5 cc. of liver emulsion in 50 cc. of the following solutions:	Sediment.
		cc.
1	Neutral control (salt solution).....	0.25
2	Weakly alkaline control (carbonate-bicarbonate).....	0.24
3	Strongly " " (0.1 N sodium hydroxide).....	Tr.
4	Neutral chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.26
5	Weakly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	0.24
6	Strongly alkaline chloramine-T, 2 per cent, equivalent to sodium hypochlorite, 0.5 per cent.....	Tr.
7	Neutral hypochlorite (sodium hypochlorite, 0.5 per cent).....	"
8	Weakly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent (Dakin's)).....	"
9	Strongly alkaline hypochlorite (sodium hypochlorite, 0.5 per cent)...	"

Experiments upon leukocytes, erythrocytes, and plasma clot in the various solutions employed in the five experiments described gave results practically identical with those obtained with liver emulsion. When, however, discs of blood clot were employed, solvent action could not be demonstrated except, possibly, to a slight degree in the strongly alkaline solutions. Blood clot is the most resistant of the substances studied against the solvent action of the solutions used.

#### DISCUSSION.

From the results recorded above, it seems justifiable to lay considerable stress on the relatively great solvent action of Dakin's hypochlorite solution as contrasted with the more recent and more stable chloramines of Dakin. It also seems probable that to its greater ability to dissolve necrotic tissue, plasma clot, and leukocytes

The changes in the glycemia are striking and are brought out by the curves of Text-fig. 1. It will be seen that the two controls showed but a slight increase in the glycemia during the 3 hours following the morphine injection, the maximum level reached being 0.15 per cent, an increase of only 0.04 per cent over the normal.

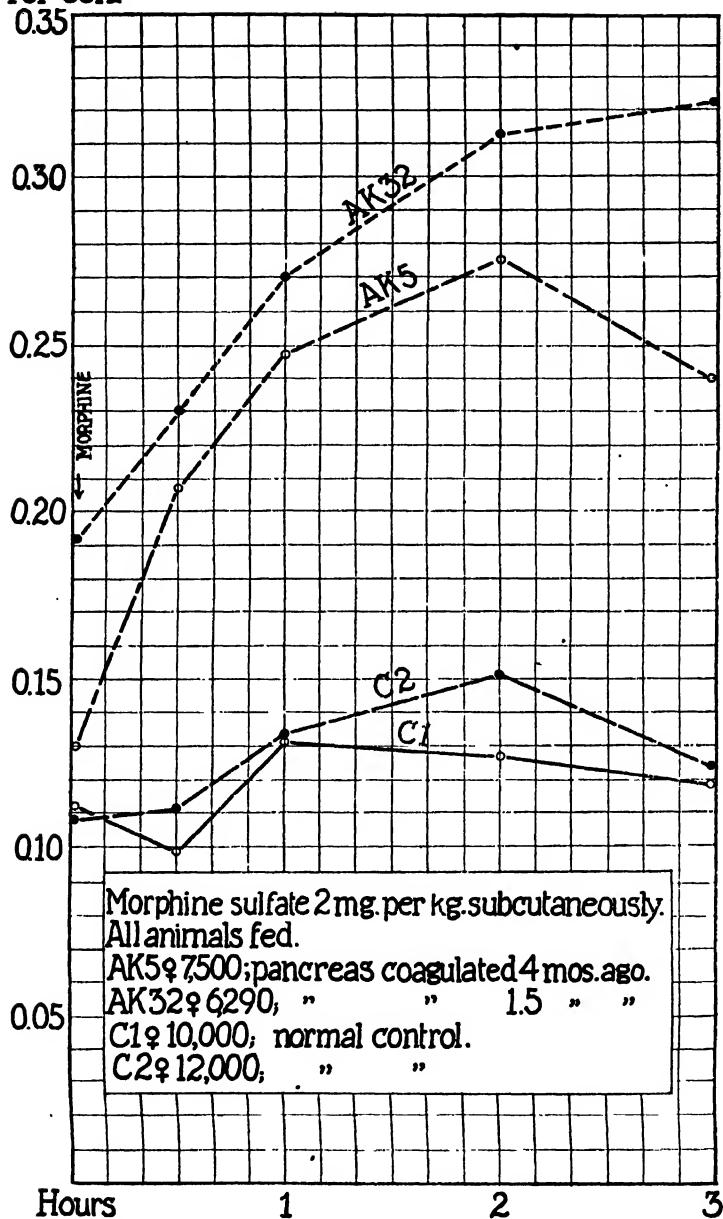
In the dogs with pancreatic deficiency, however, there was a tremendous rise in the blood sugar after the morphine. With Dog AK5 the blood sugar rose from 0.13 to 0.28 per cent after 2 hours; in Dog AK32, from 0.19 to 0.32 per cent in the same length of time. In these animals, therefore, the same dose of morphine caused a rise of 0.15 and 0.13 per cent respectively in the blood sugar, increases which are three to four times greater than those observed in the controls.

In the second series of experiments two dogs with pancreatic deficiency and two controls were employed. One of the prediabetic dogs was again Dog AK5; the second one was Dog AK37 whose pancreas with exception of the uncinate process had been resected 1 month previously, the uncinate portion with intact blood supply being transplanted to the abdominal subcutaneous tissue. The latter dog had no glycosuria beyond an occasional faint trace, and the blood sugar had ranged between 0.09 and 0.15 per cent. The controls were normal animals which had been fasted for 8 days. The prediabetic dogs, Nos. AK5 and AK37, were not fed on the day of the morphine experiment. The two controls and the dog with the subcutaneous pancreatic graft (Dog AK37) received 2 mg. of morphine sulfate per kilo subcutaneously; Dog AK5, however, was only given 1 mg. per kilo.

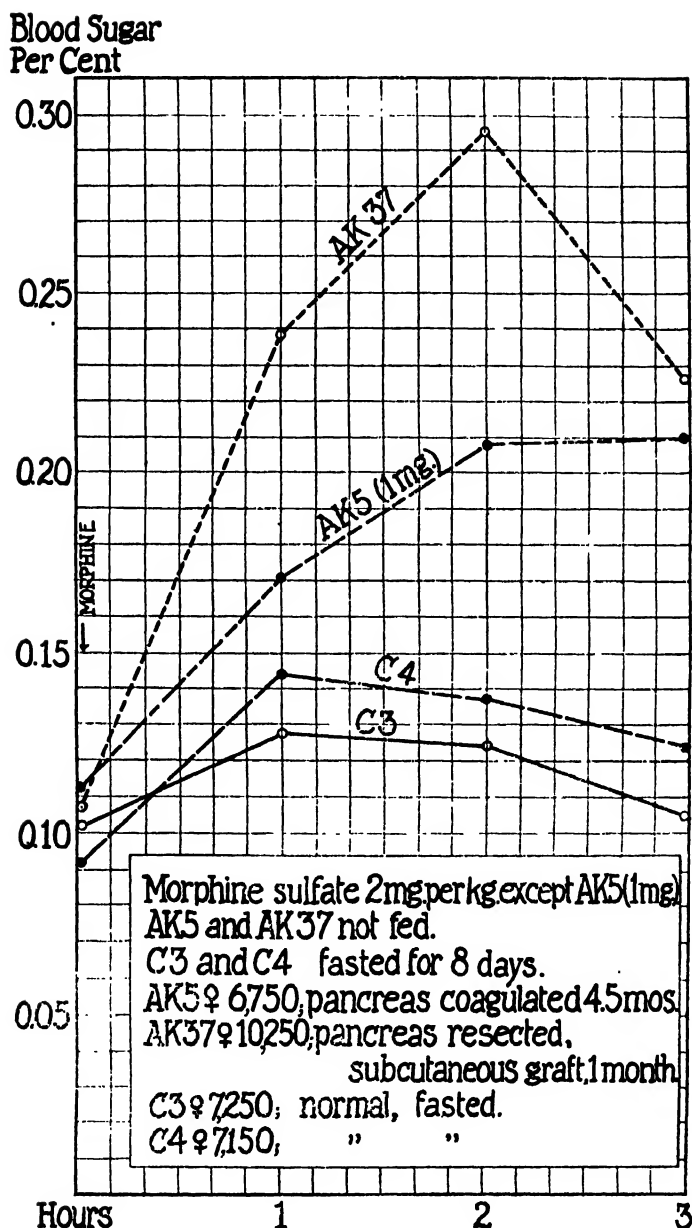
The results are clearly shown in Text-fig. 2. Here again we observe a striking quantitative difference in the glycemia of the two groups. The control dogs, starting from the 0.09 to 0.10 per cent level, show merely a rise of 0.05 per cent within 2 hours after the morphine. The prediabetic dogs, on the other hand, though beginning practically with the same glycemia as the controls, develop within 2 hours after the morphine a hyperglycemia of 0.21 per cent in Dog AK5 and 0.30 per cent in Dog AK37, levels which represent rises of 0.10 and 0.19 per cent in the blood sugar respectively. It must also be remembered that one prediabetic dog, No. AK5, received only half the amount of morphine per kilo which was given to the controls.



Blood Sugar  
Per Cent



TEXT-FIG. 1. Influence of coagulation of the pancreas on morphine glycemia.



TEXT-FIG. 2. Influence of two types of pancreatic deficiency on morphine glycemia.

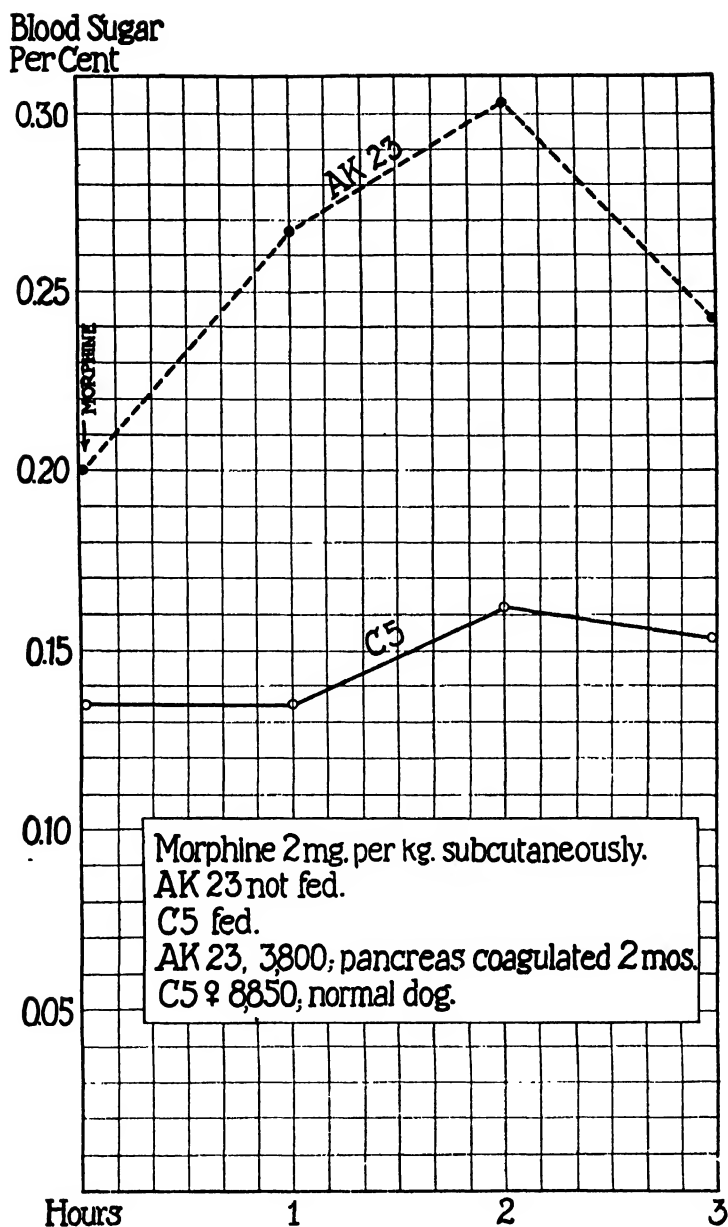
In a third group the effect of 2 mg. of morphine per kilo showed the same general difference described before, and is graphically shown in Text-fig. 3. The pancreatic deficiency in Dog AK23 was caused 2 months previously by coagulating most of the pancreas *in situ* with alcohol-acetic acid. The glycemia of this dog since operation had ranged between 0.120 and 0.20 per cent, the latter figure being reached only occasionally. There was no glycosuria except an occasional faint trace. This dog was not fed on the day of the experiment, but through an oversight the control, Dog C5, was fed 2 hours before. The latter dog vomited a large amount of food within a few minutes after the morphine was given.

From the curves of Text-fig. 3 it will be seen that the control's blood sugar rose from 0.136 to 0.161 per cent within 2 hours after the morphine administration, an increase of 0.025 per cent. The other dog, however, with deficient pancreas showed a glycemia which rose from 0.20 to 0.306 per cent within 2 hours after the morphine, a rise of 0.10 per cent, or four times more than the control.

In a fourth series we studied the effect of morphine when administered to two fasting dogs with the pancreas intact. The fasting period had lasted 22 days, the animals having free access to water. Both dogs weighed originally 8,750 gm. Dog C3 lost 2,900 gm., and Dog C4, 2,750 gm. during the fasting period. The control, Dog C6, was a normal dog weighing 7,500 gm.; it had not been fed on the day of the morphine test. The amount of morphine was 2 mg. per kilo, given subcutaneously as usual.

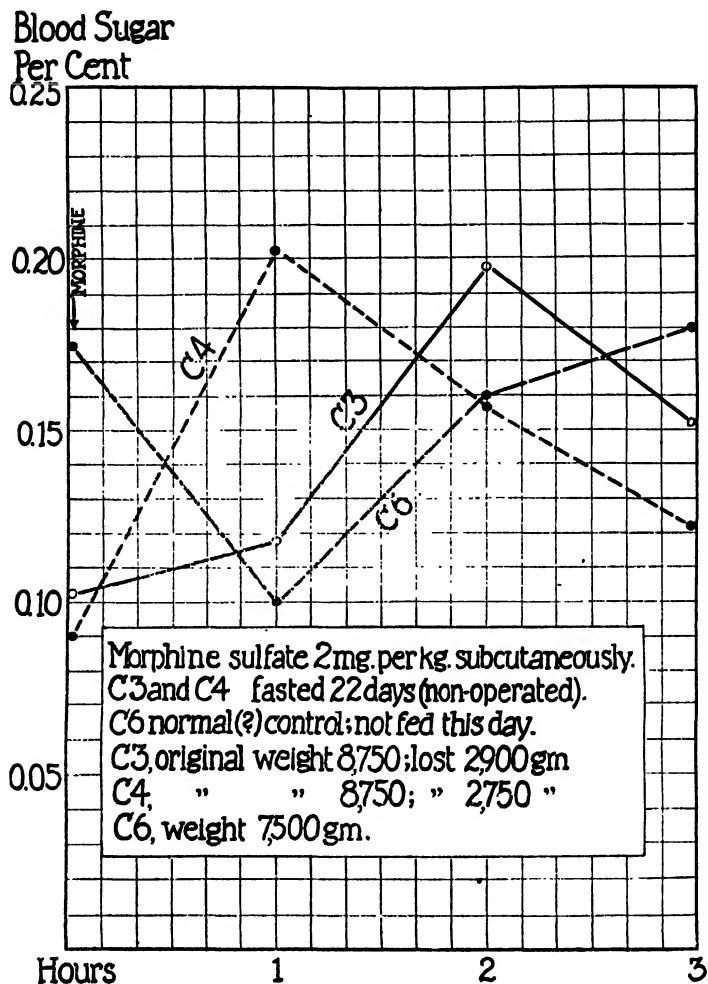
In the fasting animals, Dogs C3 and C4, the morphine produced a considerable rise in the glycemia which was fairly comparable with that observed in the dogs with an experimental pancreatic deficiency. Both dogs, starting at the normal level of 0.090 to 0.104 per cent, showed in 1 to 2 hours after the morphine test, a glycemia of 0.204 and 0.197 per cent, which represent increases of 0.09 to 0.11 per cent. The normal control, Dog C6, showed before the morphine an initially rather high glycemia, 0.175 per cent; 1 hour after the morphine the blood sugar had fallen to 0.10 per cent, but rose again during the next 2 hours to slightly above the original premorphine level. Text-fig. 4 gives these results in graphic form.

The control in this experiment can hardly be considered a normal



TEXT-FIG. 3. Influence of coagulation of the pancreas on morphine glycemia.

animal. The controls in Text-figs. 1 and 2 give a better picture of the response of a normal animal to the subcutaneous injection of morphine.



TEXT-FIG. 4. Influence of prolonged fasting on morphine glycemia in normal dogs.

In a fifth and final series of experiments we employed dogs in which a pancreatic deficiency had been produced by other means than those used in the previous groups. In Dog BD3, a female weighing 7 750

gm., five-sixths of the pancreas had been resected 3 months before, the residual sixth remaining in connection with the excretory ducts, so that the animal had some pancreatic digestion. 3 days after the operation the blood sugar was 0.277 per cent and the urine showed 0.5 per cent sugar; within a few days, however, the blood sugar fell to a normal level, fluctuating between 0.09 and 0.13 per cent, and the urine was sugar-free. In Dog AK40, a female weighing 11,000 gm., the uncinata process and the tail of the pancreas had been ligated off without resection 2 months before. As there was no reason to expect hyperglycemia or glycosuria in this animal, only one blood sugar examination was made a month after the operation; the result was 0.09 per cent. The urine was not examined. Both dogs, Nos. BD3 and AK40, were in excellent physical condition. The control dogs, Nos. C7 and C8, were apparently normal males weighing respectively 6,250 and 7,000 gm. None of the dogs were fed on the day of the morphine experiment. All the dogs of this group received 1 mg. of morphine sulfate per kilo subcutaneously in the chest.

Text-fig. 5 gives the plotted blood sugar curves of this group. The only animal which shows the characteristically prompt, strong rise in glycemia is the dog with partial pancreatectomy, No. BD3. In this dog the blood sugar rose from 0.125 to 0.213 per cent in 50 minutes, an increase of 0.09 per cent.

In Dog AK40, in which portions of the pancreas had been ligated off without resection, the rise of blood sugar did not exceed 0.03 per cent above the premorphine sample.

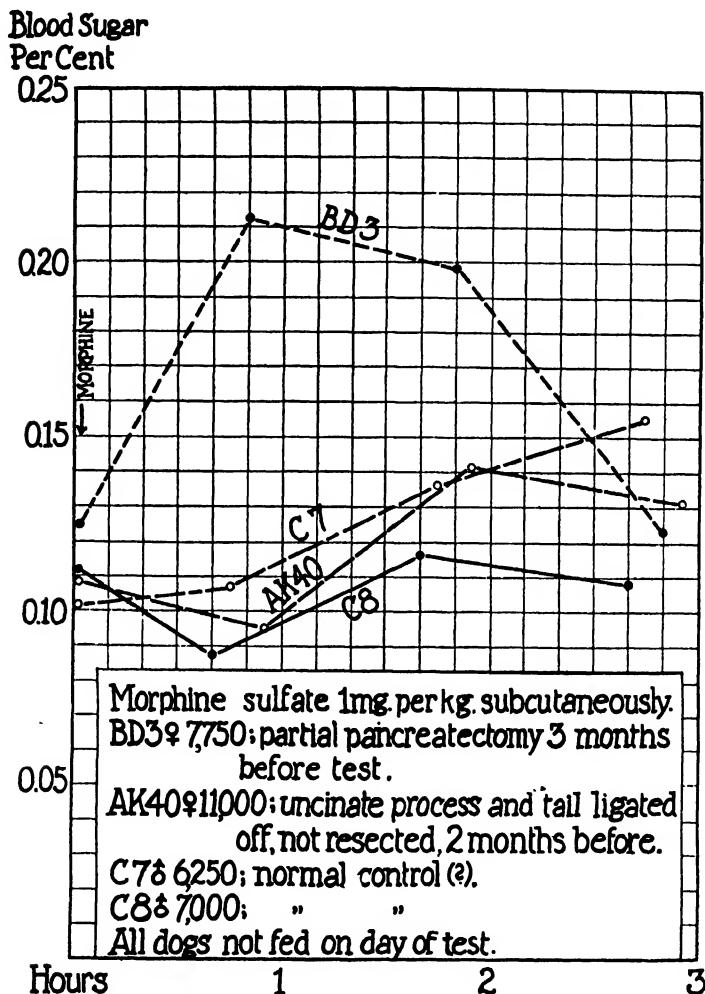
The sugar curve of the controls is quite different from that seen in Dog BD3; in Dog C7 the blood sugar rises slowly after 3 hours to 0.05 per cent above the normal level; in C8 the curve exhibits no rise whatever. The latter animal shows an initial fall of blood sugar after the injection of morphine, but in this instance the blood sample had been taken earlier than in the other dogs. A similar initial fall of the blood sugar after morphine may also be seen in Dog C1 of Text-fig. 1 where a blood sample was taken 30 minutes after the morphine dose.

*Urine.*—The urine of Dogs AK5, AK23, and AK32 (experiments of Text-figs 1 and 3) was collected for at least 12 hours after the morphine injection. No sugar was found, except in Dog AK5 (Text-fig. 1) where examination revealed

TABLE I.  
*Blood Sugar Per Cent before and after Morphine.*

Time.	Text-fig. 1.				Text-fig. 2.				Text-fig. 3.			Text-fig. 4.			Text-fig. 5.			
	Dog AK3	Dog AK32	Dog C1	Dog C2	Dog AK37	Dog AK5	Dog C3	Dog CA	Dog AK43	Dog C5	Dog C6	Dog C3	Dog CA	Dog C6	Dog AK40	Dog BD3	Dog C7	Dog C8
Before morphine. ....	0.130	0.192	0.112	0.108	0.109	0.111	0.101	0.090	0.200	0.136	0.104	0.090	0.175	—	0.109	0.125	0.104	0.114
After $\frac{1}{2}$ hr. ....	0.206	0.230	0.097	0.111	—	—	—	—	—	—	—	—	—	—	—	—	—	—
" 1 " .....	0.246	0.270	0.131	0.133	0.238	0.171	0.128	0.144	0.267	0.137	0.118	0.204	0.100	—	0.096	0.213	0.107	0.087
" 2 hrs. ....	0.275	0.315	0.128	0.151	0.297	0.209	0.124	0.138	0.306	0.161	0.197	0.157	0.160	—	0.141	0.197	0.136	0.115
" 3 " .....	0.240	0.322	0.119	0.127	0.227	0.210	0.106	0.123	0.241	0.155	0.152	0.134	0.181	—	0.130	0.124	0.157	0.109

0.52 per cent sugar in 160 cc. of urine; there was no albumin. The next 24 hour quantity of urine was sugar-free, although 28 gm. of glucose were fed daily beginning with the day after the morphine test. The urine of the other dogs, except



TEXT-FIG. 5. Influence of partial pancreatectomy on morphine glycemias.

Dog BD3 (Text-fig. 5), was not collected. Dog BD3 showed no sugar in the 18 hour urine collected after the morphine test.

*Dosage.*—The dose of morphine used in these experiments is in all probability larger than necessary. The glycemia curves of Dog AK5 in Text-fig. 2 and of



Dog BD3 in Text-fig. 5 show that a well marked rise in the blood sugar of a dog with pancreatic deficiency may be obtained with only 1 mg. of morphine per kilo. Possibly a dose of morphine can be found which will increase the blood sugar of prediabetic dogs and have no effect on the blood sugar level of normal animals.

How this hyperglycemia after morphine in dogs with a pancreatic deficiency is produced we shall not discuss here. That morphine in larger doses may cause hyperglycemia in dogs is well known.<sup>3</sup>

*General Behavior.*—The two groups of dogs of all series exhibited no marked differences in their general response to the morphine injection. All became more or less drowsy; defecation was caused in almost all animals, but retching and vomiting was practically absent in the dogs with pancreatic deficiency and in the fasted controls.

#### DISCUSSION.

The series of experiments briefly described and figured in the preceding pages show unmistakably that the subcutaneous injection of 1 to 2 mg. of morphine sulfate per kilo of body weight produces a much greater and prompter increase in the blood sugar of dogs with reduced amounts of pancreatic tissue than in normal animals. Text-figs. 1, 2, 3, and 5 and Table I illustrate this well and show the quantitative values obtained in the two classes of dogs.

There is, however, an aspect to this morphine hyperglycemia which may be of practical importance. These dogs with a small or minimal amount of pancreatic tissue may legitimately be considered in a prediabetic stage in the light of much experimental work, especially that of Allen.<sup>4</sup> On this basis, the morphine glycemia test may be of value to the clinician for detecting patients with a weakened carbohydrate metabolism, thus permitting the early institution of an appropriate dietary in order to prevent the potential diabetes from developing into actuality. The test, moreover, is easily carried out, as less than 1 cc. of blood will be necessary if the Epstein method<sup>5</sup> is employed. Only three samples of blood, each 0.2 cc. in amount,

<sup>3</sup> Hirsch, E., and Reinbach, H., *Z. physiol. Chem.*, 1914, xci, 299–301, Experiments VIII, XI, and XIV.

<sup>4</sup> Allen, F. M., *Studies concerning glycosuria and diabetes*, Cambridge, 1913..

<sup>5</sup> Epstein, A. A., *J. Am. Med. Assn.*, 1914, lxiii, 1667. A simplification of the procedure is being developed by one of us which will be ready for publication shortly.

would then be necessary, the normal, control sample, and two further samples taken 1 and 2 hours respectively after the morphine injection. The amount of morphine given to the human subject cannot, of course, be calculated kilo for kilo from the doses used for dogs; probably 20 mg. of morphine sulfate ( $\frac{1}{3}$  grain) would suffice for an adult.

Whether the morphine test will yield the same result with human beings in the prediabetic stage which we obtained experimentally in dogs, only actual trial can determine. Such a trial, however, we believe warranted by our results and by the simplicity of the procedure. The injection of a moderate dose of morphine is surely not more of a strain to the organism with a possibly defective carbohydrate metabolism than the ingestion of 100 to 200 gm. of glucose; moreover, it will be remembered that morphine has been and is administered to diabetics with apparently beneficial results. Thus, for example, Pavy<sup>6</sup> reported that opium, morphine, and especially codeine reduce the glycosuria in human diabetes. It is therefore unlikely that morphine will work harm in the prediabetic stage of human diabetes.

When to suspect the prediabetic stage in a patient will offer no difficulties to the physician. The combination of racial or family predisposition, neurotic temperament, rheumatoid pains, and periods of muscular weakness point to a possibly defective carbohydrate metabolism, though not associated with a glycosuria. If there is furunculosis, or pruritus, or increased thirst and micturition, or early development of impotence, a prediabetic stage is to be suspected, even though the urine is sugar-free. In such cases the morphine test is worthy of a trial.

*Sugar Tolerance.*—It should be mentioned that the sugar tolerance of the dogs in which the pancreas had been coagulated by alcohol was surprisingly good. For example, Dog AK5, 90 days after operation excreted only 0.3 gm. of sugar per kilo after being fed 10 gm. per kilo. From the 119th day to the 175th day the same dog was fed daily, except on the day of the morphine test, 4 gm. of sugar per kilo,

<sup>6</sup> Pavy, F. W., *Guy's Hosp. Rep.*, 1870, xv, series 3, 420. It may be noted that as much as 2½ grains (165 mg.) of morphine hydrochloride were administered three times a day to Pavy's Case 3 (p. 430).

in addition to the regular mixed diet, but no sugar appeared in the 24 hour urines.

Another example is furnished by Dog AK32. This dog showed a severe diabetes during the 1st week after the operation: the glycosuria varied between 2.7 to 4.8 per cent and the blood sugar ranged from 0.16 to 0.32 per cent. Within 2 weeks the urine became sugar-free and the glycemia oscillated between 0.09 and 0.17 per cent. A tolerance test on the 21st day after operation (10 gm. of sugar per kilo *per os*) caused no sugar excretion whatever. Thereafter this animal's urine up to the time of death, 118 days after operation, never showed any sugar, beyond an occasional faint trace. It is therefore evident that a strong hyperglycemia after small doses of morphine can even then be obtained when the carbohydrate metabolism is only moderately impaired.

*Fasting.*—In dogs which had fasted sufficiently long, the subcutaneous injection of 2 mg. of morphine sulfate per kilo sufficed apparently to bring on a definite hyperglycemia. This is shown in Text-fig. 4. In the two animals, Dogs C3 and C4, which had fasted 22 days, the subcutaneous injection of 2 mg. of morphine sulfate per kilo produced in 1 to 2 hours a hyperglycemia of 0.20 per cent, the normal level of the same animals being 0.09 to 0.10 per cent. Since the dogs with deficient pancreas, excepting Dog BD3 (Text-fig. 5), were losing weight constantly in spite of a liberal mixed diet, because they were devoid of pancreatic digestion, it might be objected that the morphine hyperglycemia which we have described merely indicates a fasting state and not a weakness of the carbohydrate metabolism, as we have assumed. This interpretation, however, is probably only partially correct at best and by no means decreases the value of our results. It has been well known since the time of Claude Bernard that fasting or cachectic animals in general may respond with a transitory glycosuria to the ingestion of a full meal of carbohydrates, but it is clear that such an alimentary glycosuria must be due to a temporarily weakened carbohydrate metabolism, for the normal organism would show a sugar-free urine. Therefore the morphine hyperglycemia which we observed during fasting is additional evidence for the correctness of our working hypothesis that morphine

will cause a greater hyperglycemia in an animal with impaired carbohydrate metabolism than in a normal individual.

On the whole, therefore, it may be said that the morphine hyperglycemia during severe fasting is not only no evidence against the correctness of our hypothesis but is, on the contrary, just what that view demands. Furthermore, it must be emphasized that a pancreatic deficiency without obvious fasting, as in Dog BD3 (Text-fig. 5), also causes a well marked hyperglycemia when a small dose of morphine is injected subcutaneously; severe fasting *per se*, therefore, is also not a necessary factor for the appearance of a marked hyperglycemia after morphine in prediabetic dogs.

It should be observed that a moderate degree of fasting is insufficient to bring out the morphine hyperglycemia. Thus a fasting period of 8 days in Dogs C3 and C4 did not cause a marked hyperglycemia after morphine, as the curves of Text-fig. 2 show.

#### SUMMARY.

The subcutaneous injection of 1 or 2 mg. of morphine sulfate per kilo subcutaneously in dogs with a pancreatic deficiency, whose sugar tolerance is still good, produces a rise in the glycemia about four times greater than the same amount of morphine calls forth in normal dogs.

As dogs with a pancreatic deficiency due to coagulation or partial resection of the gland may legitimately be considered in a prediabetic state, the inference is warranted that the morphine test may be of value in detecting a weakened carbohydrate metabolism in the human subject. The test could easily and without danger be carried out with the micro methods now available for the quantitative determination of blood sugar.

The experimental facts described in this paper give additional corroboration to the view that the response of a normal and of a pathologically altered organism to the same drug in the same dosage may be quantitatively very different.



## LECITHIN. I.

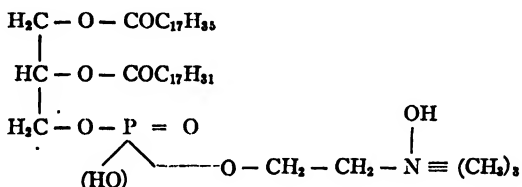
### "HYDROLECITHIN" AND ITS BEARING ON THE CONSTITUTION OF CEPHALIN.

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The recent investigations on the chemical structure of lecithin have resulted in many important contributions, all of which point to the correctness of the generally accepted view of its molecular structure,



However, a scrutiny of all the work on lecithin reveals a remarkable incompleteness of each individual investigation. A rigorous proof of the accepted theory requires for lecithin an elementary composition of C = 65.60, H = 10.79, N = 1.74, P = 3.86. It further requires that the nitrogen of the molecule should be composed entirely of choline. Hence, lecithin should not contain even a part of its nitrogen in the form of free amino groups. Still further, it requires a proof of the identity of the fatty acids with those accepted by theory, and finally it requires the isolation of the glycerophosphoric acid.

The work up to the present has satisfied many of the requirements. The fatty acids and the glycerophosphoric acid have been identified; and MacLean has prepared, at least once, a sample of lecithin that was free of amino nitrogen. However, this one sample has been incompletely analyzed. All other samples of lecithin prepared by various workers contained amino nitrogen in their molecule, and

from the standpoint of elementary analysis showed a marked disagreement with the theory.\* This is well illustrated by the following table.

Author.	Source.	C	H	N	P	Choline
		per cent	per cent	per cent	per cent	per cent
Thudichum <sup>1</sup> .....	Brain.	66.75	18.67	1.81	4.00	—
Baskoff <sup>2</sup> .....	Liver.	64.64	10.71	1.95	4.00	—
Heffter <sup>3</sup> .....	"	—	—	—	—	25
Stern and Thierfelder <sup>4</sup> .....	Egg.	64.63	10.96	1.79	3.95	—
MacLean <sup>5</sup> .....	"	64.18	10.60	1.87	3.95	66
Erlandsen <sup>6</sup> .....	Heart.	66.29	10.17	1.87	3.95	42
MacLean <sup>7</sup> .....	"	66.27	10.32	1.85	3.97	41.4
Eppler <sup>8</sup> .....	"	66.46	10.69	1.87	4.03	—
MacLean <sup>7</sup> .....	"	—	—	1.89	4.04	68
".....	Not given.	—	—	1.85	4.00	66
".....	From CdCl <sub>2</sub> salt.	—	—	1.87	4.15	98.7

The analytical data obtained by Ritter<sup>9</sup> on hydrolecithin showed better agreement with the theory, and one might be inclined to regard the material of Ritter as such that it contained all the necessary proof in favor of the conventional theory. Unfortunately, Ritter did not determine the amino nitrogen content of the reduced lecithin, and hence failed to furnish definite proof of its purity.

Indeed, the present report contains data unmistakably proving that hydrolecithin of an elementary composition fully harmonizing with the theory may be and generally is impure, containing between 10 and 20 per cent of nitrogen in the form of amino nitrogen. Thus the task of the preparation of lecithin having a composition required by the theory and at the same time free of impurities has not yet been

<sup>1</sup> Thudichum, J. L. W., *The chemical constitution of the brain*, London, 1884.

<sup>2</sup> Baskoff, A., *Z. physiol. Chem.*, 1907, lvii, 395.

<sup>3</sup> Heffter, A., *Arch. exp. Path. u. Pharm.*, 1891, xxviii, 100.

<sup>4</sup> Stern, M., and Thierfelder, H., *Z. physiol. Chem.*, 1907, liii, 381.

<sup>5</sup> MacLean, H., *Z. physiol. Chem.*, 1908, lv, 360; 1909, lix, 223; *Biochem. J.*, 1909, iv, 38, 240.

<sup>6</sup> Erlandsen, A., *Z. physiol. Chem.*, 1907, li, 71.

<sup>7</sup> MacLean, *Biochem. J.*, 1915, ix, 364.

<sup>8</sup> Eppler, J., *Z. physiol. Chem.*, 1913, lxxxvii, 241.

<sup>9</sup> Ritter, F., *Ber. chem. Ges.*, 1914, xlvii, 530. Cf. *Reidel's Ber.*, 1913, lvii, 20; 1914, lviii, 15.

accomplished. Efforts in this direction are now in progress in this laboratory.

However, the present finding has a great significance because of its bearing on the structure of cephalin, and the work is presented in its present incomplete state because of this significance. The remarks made earlier in this communication regarding lecithin apply also to cephalin. On the basis of recent work on the hydrolytic products of the substance, a certain structural formula has been assumed. This formula requires an elementary composition of  $C = 66.17$ ,  $H = 10.57$ ,  $N = 1.88$ , and  $P = 4.17$ . However, all samples analyzed, beginning with Thudichum and up to the present by the most recent investigators,<sup>10</sup> consistently had the average composition of  $C = 60.00$ ,  $H = 9.30$ ,  $N = 1.80$ , and  $P = 3.80$ .

On the basis of these considerations, one may argue that if cephalin and lecithin both had the composition required for them by the theory, then a mixture of the two should possess practically the same elementary composition as either one of them in the pure state. On the other hand, if lecithin possessed the composition assumed by the theory and cephalin that found empirically, then a mixture containing 80 per cent of the former and 20 per cent of the latter should possess a carbon content of 64.56 per cent instead of 65.35 per cent. Conversely, if a mixture of the two reduced substances possessed an elementary analysis of  $C = 65.30$ ,  $H = 11.20$ ,  $N = 1.75$ ,  $P = 3.85$ , it would justify the conclusion that both lecithin and cephalin possess the composition assumed for them by the theory.

The material analyzed by us contained 80 per cent of lecithin and 20 per cent of an impurity. It was found that the material yielded on hydrolysis besides the choline also the base amino-ethanol. Hence it was reasonable to assume that the 20 per cent of impurity consisted of cephalin. If cephalin had the composition found by experience then a substance consisting of 80 per cent of hydrolecithin and 20 per cent of cephalin should have an elementary composition of  $C = 64.56$ ,  $H = 10.49$ ,  $N = 1.75$ ,  $P = 3.84$ . On the other hand, if both lecithin and cephalin possess the structure assigned to them by theory

<sup>10</sup> Levene, P. A., and West, C. J., *J. Biol. Chem.*, 1916, xxiv, 41.



then the above mixture should have the elementary composition found by experiment. Thus the facts presented in this report furnish evidence in favor of the prevailing theory of the molecular structure of lecithin and of cephalin; they also indicate the method by which the pure reduced cephalin may eventually be obtained. Efforts in this direction are now in progress.

In this connection it may be recalled that the product obtained on reduction of cephalin with hydrogen gas in the presence of palladium contained from 62 to 63 per cent of carbon in its molecule, thus approaching nearer than the non-reduced cephalin the theoretical value. The non-reduced cephalin generally obtained is undoubtedly an altered and perhaps oxidized form of the original substance. The nature of the alteration is not known as yet.

#### EXPERIMENTAL.

Hydrolecithin was first prepared by Paal and Oehme;<sup>11</sup> they reduced an alcoholic solution of egg lecithin with hydrogen and colloidal palladium. The product was obtained as microscopic compact crystals, which sintered at 83–84°, and decomposed over 150° with blackening. Upon hydrolysis the hydrolecithin gave a mixture of stearic, palmitic, and probably myristic acids. The following year Ritter<sup>8</sup> reduced lecithin which had been prepared from fresh, dry egg yolk as follows: The egg yolk was first extracted with petroleum ether and then with ether; the ethereal extract was concentrated and the residue was then extracted with methyl alcohol. The hydrolecithin prepared in this way proved to be distearylhydrolecithin; that is, on hydrolysis it yielded only stearic acid.

Hydrolecithin has been prepared in our laboratory, by Paal's method, from various fractions of egg lecithin, and from lecithin of brain and other organs. During the course of the work it has been noted that lecithin, which has been washed according to MacLean's<sup>7</sup> method, in which the lecithin is ground up with a little water and precipitated with acetone, is reduced more rapidly and more completely than unwashed lecithin. Also, it was found that the addition of 1 to 2 per cent of acetic acid to the alcoholic solution facilitated

<sup>11</sup> Paal, C., and Oehme, H., *Ber. chem. Ges.*, 1913, xlv, 1297.

the reduction. With fairly concentrated solutions the hydrolecithin separates out during the course of the reduction. This product was brought into solution by warming, filtered from the coagulated palladium, cooled to  $0^{\circ}$ , and the material which separated out crystallized from dry methyl ethyl ketone until the composition was constant. In most cases it was easily possible to obtain material with correct analytical figures for carbon and hydrogen. In some cases, however, this was impossible, even after repeated crystallization.

Hydrolecithin crystallizes well from methyl ethyl ketone, in which it is insoluble in the cold; it softens between  $80$  and  $90^{\circ}$ , turns brown about  $100^{\circ}$ , starts to melt about  $200^{\circ}$ , and runs down the tube, giving a dark red liquid, at  $235^{\circ}$ . The figures vary somewhat, depending upon the rate of heating. The optical activity was determined in chloroform solution and varied between  $+5.2^{\circ}$  and  $+5.4^{\circ}$ . The presence of the amino nitrogen-containing body did not seem to affect the value for  $[\alpha]_D^{20}$ .

$$\text{Sample 400 } [\alpha]_D^{20} = \frac{9.7822 \times 0.18^{\circ}}{0.5 \times 0.3254} = +5.4^{\circ}$$

$$\text{" 399 } [\alpha]_D^{20} = \frac{9.2784 \times 0.21^{\circ}}{0.5 \times 0.3734} = +5.22^{\circ}$$

$$\text{" 429 } [\alpha]_D^{20} = \frac{9.2150 \times 0.24^{\circ}}{0.5 \times 0.4170} = +5.3^{\circ}$$

$$\text{" 457 } [\alpha]_D^{20} = \frac{9.330 \times 0.23^{\circ}}{0.5 \times 0.4120} = +5.20^{\circ}$$

Analysis of these samples gave the following values:

Sample.	C	H	N	P	Ash	NH <sub>2</sub> -N
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
400	65.20	10.89	2.00	4.00	10.61	
399	65.59	11.16	2.37	3.85	10.38	
429	65.60	11.03	1.96	3.79	9.75	7.00
508	65.50	11.30	1.80	3.90	—	20.00
492	—	—	1.98	3.98	9.66	6.00
Theory.	65.37	11.23	1.74	3.84		0

*Hydrolysis of Hydrolecithin.*

A large quantity (200 gm.) of hydrolecithin was prepared for the purpose of studying its hydrolytic products. This analyzed as follows:

Sample.	C	H	N	P	NH <sub>2</sub> -N
508	65.50	11.30	1.80	3.90	20.00

100 gm. of this material were hydrolyzed by boiling with 1 liter of 3 per cent sulfuric acid for 8 hours.<sup>7</sup>

The fatty acid fraction was filtered off and recrystallized from acetone, after boiling about 2 hours with animal charcoal. Again recrystallized from acetone, the acid melted at 69–70°, and on combustion and titration gave figures for *stearic acid*. This confirms Ritter's observation that it is possible to obtain pure distearyl-hydrolecithin.

The aqueous filtrate was freed from sulfuric acid by the addition of barium hydroxide, concentrated *in vacuo*, precipitated with basic lead acetate, the filtrate freed from lead, and used for the determination of aminoethyl alcohol according to Thierfelder and Schulze.<sup>12</sup> The ethereal extract was concentrated, taken up in water, and the amino nitrogen determined. The theoretical amount of gold chloride<sup>4</sup> was added to the acidified solution. The gold salt separated as long needles after standing 2 days in a desiccator over sulfuric acid. It melts at 184–186°. Trier<sup>13</sup> gives 186–187° for the gold chloride

<sup>12</sup> Thierfelder, H., and Schulze, O., *Z. physiol. Chem.*, 1916, xcvi, 296.

This method depends upon the fact that calcium oxide does not liberate choline from its hydrochloride, but does free aminoethyl alcohol. The concentrated solution of the mixed hydrochlorides is rubbed up with pure calcium oxide until it is dry powder and extracted with ether in a Soxhlet apparatus; the flask should contain 0.1 N sulfuric acid to bind the base, otherwise considerable loss occurs. After 27 hours about 96 per cent of the alcohol has been extracted by the ether. The choline may then be extracted with hot alcohol.

<sup>13</sup> Trier, G., *Z. physiol. Chem.*, 1911, lxxiii, 383; 1911–12, lxxvi, 496.

salt of aminoethyl alcohol hydrochloride, and Knorr<sup>14</sup> gives about 190° for the synthetic product. It was analyzed by heating to constant weight.

0.2008 gm. substance gave 0.0990 gm. Au.

0.2075 " " " 0.1016 " "

	Calculated:	Found:	
Au.....	49.17	49.35	48.96

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<sup>14</sup> Knorr, L., *Ber. chem. Ges.*, 1897, xxx, 913.



## THE STRUCTURE OF YEAST NUCLEIC ACID.

### II. URIDINEPHOSPHORIC ACID.

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

#### PLATE 1.

(Received for publication, December 1, 1917.)

In a series of articles published in the course of the last few years, Walter Jones<sup>1</sup> and his coworkers advanced a theory on the mode of linkage of the four nucleotides taking part in the molecular structure of yeast nucleic acid. According to these authors, the nucleus of yeast nucleic acid is a tetra-ribose of the following structure  $[(C_5H_{10}O_5)_4 - 3 H_2O]$ . Jones and his coworkers have based their conclusions on the analysis of three substances which they had regarded as dinucleotides.

In a previous publication<sup>2</sup> it was pointed out that the theory of Jones and his coworkers was not the only possible conclusion from the facts presented by them. It was further pointed out that not sufficient rigor had been exercised in proving the dinucleotide structure of the substances described by them. In the same publication the present author reported in a preliminary way the results of his own attempts to fractionate the pyrimidine nucleotides described by Levene and Jacobs.<sup>3</sup> For this purpose the crude nucleotides were transformed into the brucine salts and these were repeatedly recrystallized from dry methyl alcohol. In this manner a substance of constant composition was obtained. On conversion of the brucine

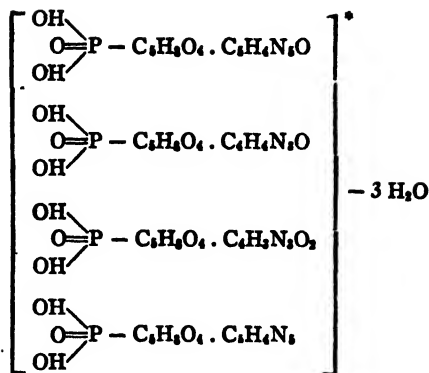
<sup>1</sup> Jones, W., and Richards, A. E., *J. Biol. Chem.*, 1914, xvii, 71. Jones, W., and Germann, H. C., *ibid.*, 1916, xxv, 93. Jones, W., and Read, B. E., *ibid.*, 1917, xxix, 123; xxxi, 39.

<sup>2</sup> Levene, P. A., *J. Biol. Chem.*, 1917, xxxi, 591.

<sup>3</sup> Levene, P. A., and Jacobs, W. A., *Ber. chem. Ges.*, 1911, xlv, 1027; *J. Biol. Chem.*, 1912, xii, 411.

salt into a barium salt, the composition of the substance was not altered. Both the brucine and the barium salt had the elementary composition of a dinucleotide. And yet, such a conclusion did not appear entirely compelling, and further attempts at fractionation seemed desirable. Crystallization of the same crude brucine salts from a 35 per cent solution of ethyl alcohol was now resorted to. Passing the salts through eight recrystallizations, it was possible to separate them into two principal fractions. The less soluble part had a composition of the salt of uridinephosphoric acid and the more soluble that of cytidinephosphoric acid. Each of these could be converted into its barium salt. The barium salt of the uridinephosphoric acid precipitated out of a concentrated aqueous solution in the form of long needles, aggregated in rosettes resembling in form those of an osazone. On the other hand, the barium salt of the cytidinephosphoric acid appeared under the same conditions in the form of microscopic granules. The two salts differed in their solubility and in their optical rotation. In this communication only the results of the analysis of uridinephosphoric acid will be reported since attempts are still in progress to obtain the barium salt of the cytidinephosphoric acid also in crystalline form. The purity of the uridinephosphoric acid has been proven by the fact that amino nitrogen could not be detected on the analysis, either of the barium salt directly, or after previous hydrolysis. Nor was it possible to isolate from the product of its hydrolysis any other base than uracil. The specific rotation of the substance was  $[\alpha]_D^{20} = +3.5^\circ$ .

Thus it is evident that the substance previously described as pyrimidine dinucleotide was a mixture of two mononucleotides. This observation is important inasmuch as it demonstrates conclusively that for the present there is no experimental proof for the assumption of a tetra-ribose as the nucleus of yeast nucleic acid. This, however, does not exclude the possibility that such proof may be furnished in the future. Meanwhile the structural formula of yeast nucleic acid, free from all arbitrary elements, may be written in the following form.



## EXPERIMENTAL.

The condition of hydrolysis and the method of preparation of the mixed pyrimidine nucleotides were the same as previously described. Care was taken to keep the temperature of the oil bath during the hydrolysis at 100°C.

The crude silver salts were suspended in water and freed from silver by means of hydrogen sulfide. The filtrate from silver sulfide was freed from hydrogen sulfide by aeration, then rendered alkaline by means of a solution of barium hydroxide in order to remove the phosphoric acid. The filtrate was then neutralized and concentrated at diminished pressure and at 50°C. From the concentrated solution the barium was removed quantitatively and to the filtrate brucine in methyl alcoholic solution was added until the originally acid solution turned slightly alkaline to litmus. On standing, a crystalline deposit of the nucleotides formed.

The separation of the two nucleotides was brought about by recrystallization from 35 per cent ethyl alcohol. After eight recryst-

\* Bottomley has recently (*Proc. Roy. Soc., B*, 1917, xc, 39) criticized the view expressed by Levene and Medigreceanu (*J. Biol. Chem.*, 1911, ix, 375, 389) that the first phase in the enzymatic cleavage of nucleic acid consisted in the dissolution of the union between individual mononucleotides; Bottomley modified the theory, accepting that the first phase of decomposition of nucleic acid is limited to its cleavage into two dinucleotides. This hypothesis may eventually prove correct; however, the evidence furnished by Bottomley is unsatisfactory. The author made no attempt to fractionate his crude material. For the present the conclusion of Bottomley seems to us unsustained by facts.



tallizations the brucine salt of a pure uridinenucleotide was obtained. In the purification of the brucine salt one may, to some extent, be guided by its melting point. The pure material on heating in a capillary tube first contracts at 183°C. (corrected), then melts, and finally decomposes at 198°C. (corrected). However, even after this phase is attained it is advisable to repeat recrystallization at least three times. The optical rotation of the substance was not measured because of its great insolubility in water and in other solvents. The solubility of the salt is greater in dilute alcohol than in water, but the solubility even in this reagent is not great enough to permit an accurate optical measurement.

The composition of the brucine salt (No. 70) was the following:

0.1027 gm. substance gave 0.1998 gm. CO<sub>2</sub> and 0.0584 gm. H<sub>2</sub>O.  
 0.2000 " " " 12.0 cc. of nitrogen at t° = 22°C. and p = 764 mm.  
 0.2000 " " " 0.0168 gm. Mg<sub>3</sub>P<sub>2</sub>O<sub>7</sub>.

	Calculated for C <sub>26</sub> H <sub>32</sub> N <sub>4</sub> PO <sub>7</sub> + 7H <sub>2</sub> O:	Found:
C.....	53.20	53.06
H.....	6.51	6.37
N.....	6.80	6.96
P.....	2.52	2.37

*Conversion of the Brucine Salt into the Barium Salt.*—The brucine salt was dissolved in hot water by means of an excess of ammonia water, and the brucine was extracted by means of chloroform in a separating funnel. The aqueous solution of the nucleotide was repeatedly evaporated to dryness under diminished pressure with an excess of barium hydroxide until all ammonium was removed. The residue was then dissolved by the aid of some sulfuric acid, and to the solution a barium hydroxide solution was added until the reaction turned slightly alkaline to phenolphthalein. The filtrate from the barium hydroxide was concentrated under diminished pressure at a temperature of the water bath not exceeding 50°C. It is preferable to interrupt the distillation just before the nucleotide begins to settle out while the distillation is still in progress, since in such case it may have an amorphous appearance. If desired the barium salt may be recrystallized out of water. When air-dry the substance has a granular appearance, and microscopically it consists of rosettes composed of long needles, as is shown in the figures.

## Sample 57.

0.1024 gm. substance gave 0.0888 gm.  $\text{CO}_2$ , 0.0242 gm.  $\text{H}_2\text{O}$ , and 0.0502 gm.  $\text{Ba}_3\text{P}_2\text{O}_7$ .

0.1015 gm. used for Kjeldahl nitrogen estimation required for neutralization 4.47 cc. 0.1 N acid.

## Sample 55.

0.1422 gm. substance used for Kjeldahl nitrogen estimation required for neutralization 6.36 cc. 0.1 N acid.

0.1422 gm. substance gave on fusion 0.0348 gm.  $\text{Mg}_3\text{P}_2\text{O}_7$ .

0.0711 " " " 0.0356 gm.  $\text{BaSO}_4$ .

	Calculated for $\text{C}_6\text{H}_8\text{N}_4\text{O}_8\text{P}_2\text{Ba}$ :	No. 55	Found: No. 57
C.....	23.50		23.65
H.....	2.41		2.64
N.....	6.10	6.27	6.16
P.....	6.75	6.82	
Ba.....	29.90	29.47	
$\text{Ba}_3\text{P}_2\text{O}_7$ .....	48.97		49.02

The specific rotation of the Ba salt in a 2.5 per cent solution of HCl was

$$[\alpha]_D^{25} = \frac{+0.14^\circ \times 100}{1 \times 4} = +3.5^\circ.$$

*Hydrolysis of Barium Salt.*—9.0 gm. of the barium salt were dissolved in 100.0 cc. of water to which 13.0 gm. of sulfuric acid were added and the solution was heated for 4 hours at  $125^\circ\text{C}$ . in a sealed tube. The sulfuric acid was removed quantitatively and the solution was concentrated under diminished pressure until uracil began to crystallize. The mother liquor from uracil was tested for amino nitrogen with a negative result. Also the attempt to prepare an insoluble picrate of cytosine was unsuccessful.

The uracil was recrystallized from dilute sulfuric acid and had the following composition:

0.0984 gm. substance was employed for a Kjeldahl nitrogen estimation and required for neutralization 17.49 cc. of 0.1 N acid.

	Calculated for $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$ :	Found:
N.....	25.05	24.88

*Cytidinephosphoric Acid.*—The mother liquor from the brucine salt of the uridinephosphoric acid was concentrated until a second

crystallization began to deposit. This second brucine salt was converted into a barium salt, which had the composition of the salt of cytidinephosphoric acid. The salt deposited in form of microscopic globules. Attempts are being made to obtain it in a crystalline form and, because of this, the results of the analysis of the substance are deferred to a later date.

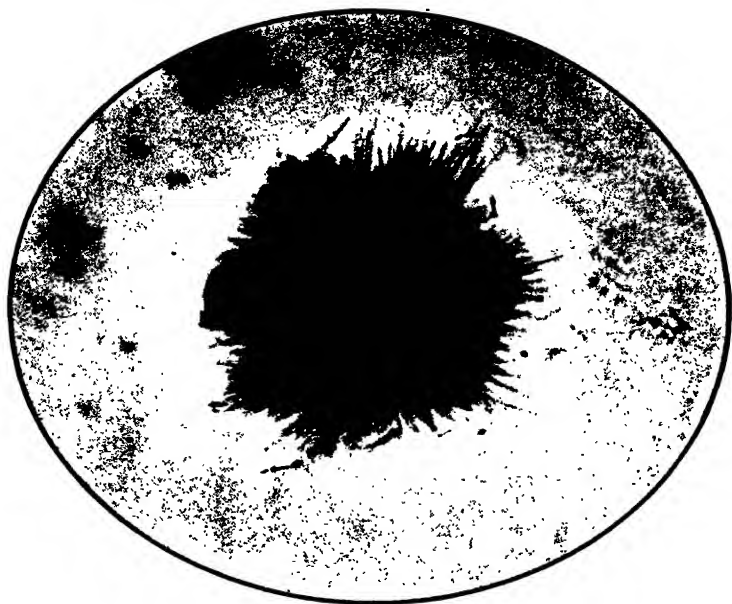


FIG. 1.



FIG. 2.

(Levene: Yeast nucleic acid II.)



## NOTE ON RAPID ORGANIC COMBUSTION.

By P. A. LEVENE AND F. W. BIEBER.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

We wish to report here on a method for rapid combustion as it is practiced in our laboratory. There is nothing essentially new in the principle of the method as employed by us. To Dennstedt,<sup>1</sup> to Beck<sup>2</sup> and to Miss Marie Reimer<sup>3</sup> belong the credit of building up the theory and practice of the rapid combustion. However, in our laboratory where the demand made on every furnace is very great and where combustions are made continually, there were many occasions when none of the three methods worked satisfactorily, even when carried out exactly under the original conditions given by their respective authors. In the course of years some of the imperfections of the three methods were corrected, and the advantageous parts of all were combined in one, so that finally we have a combustion tube which served for more than three hundred combustions without change of the catalyst.

Cerium dioxide was employed as the catalyst, as suggested by Beck. It was soon found that when the dioxide was prepared in the manner described by the original authors, samples were obtained that differed considerably in their efficiency. Often the catalyst was exhausted after a very few combustions. On other occasions the dioxide was inactive from the start, and at times an inactive dioxide improved after several combustions, but became exhausted after a few additional combustions. As the same source of cerium nitrate gave samples of dioxide of variable efficiency it was thought that some component of illuminating gas had a deleterious effect on the catalyst. Because of this it was concluded to convert the nitrate into the dioxide in an atmosphere of oxygen. The catalyst prepared in this manner seems to maintain its efficiency indefinitely.

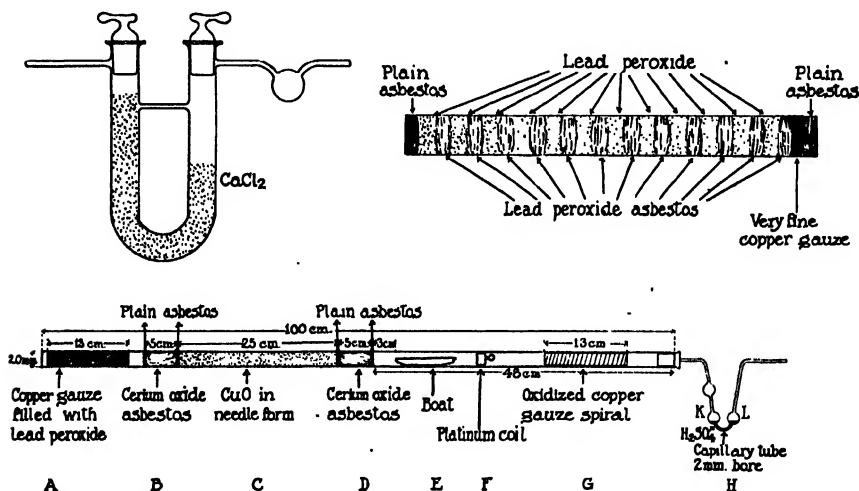
<sup>1</sup> Dennstedt, M., "Anleitung zur vereinfachten Elementaranalyse," III. Aufl. Hamburg, 1910, p. 66.

<sup>2</sup> Beck, J., *Ber.*, **46**, 2574 (1913).

<sup>3</sup> Reimer, M., *THIS JOURNAL*, **37**, 1636-38 (1915).

The central part of the tube is filled with copper oxide in wire form. At each end of the copper oxide is placed a charge of cerium dioxide. There is a layer of pure asbestos between the copper oxide and the catalyst, as well as between the catalyst and the neighboring part. An innovation in the tube is a small coil made of very fine platinum gauze. The coil serves as a protection for the boat from copper oxide that might be carried over from the terminal copper coil. Through the presence of this coil it is possible to obtain reliable data, regarding the ash content of a substance.

The manner of filling the tube is best seen from the accompanying diagrams.



The combustion tubes should be 100 cm. long and 20 mm. in diameter. When the demand on the furnace is considerable, a tube of transparent quartz was found economical. The combustion tube is connected to any conventional drying train by means of part H. This consists of a capillary tube of 2 mm. bore and of three bulbs, 15 mm. in diameter. The distance between K and L is 25 mm. The section is filled with sulfuric acid.

**Absorption Apparatus.**—For the absorption of water a U-tube, provided with a bulb for retaining the condensed water as given on the drawing, is recommended. For the absorption of carbon dioxide two usual U-tubes filled with soda lime are employed.

*The lead peroxide* is kept in position in a copper gauze wrapper. Layers of pure peroxide alternate with layers of asbestos impregnated with the reagent. For this purpose, the asbestos is mixed with an equal volume of dry peroxide.

*Catalyst.*—Purified asbestos fiber is suspended in a saturated solution of cerium nitrate, and the mixture is placed on a boiling water bath to remove the greater part of the water. The still moist asbestos is then transferred to a glass tube and is heated in a stream of oxygen. One end of the tube is drawn to a narrow point which is connected with the oxygen supply. The opposite end is provided with a stopper containing a glass tube, which dips into a flask containing dilute alkali.

*Rules for Combustion.*—The burners under Section A of the tube are regulated so that the temperature in the center of the lead peroxide is kept constant at 300 to 320°. This temperature should be regulated when all the burners are lighted so as to give the maximum heat required by the combustion. Once so adjusted, the burners remain fixed and should not be disturbed so long as the same charge of the tube remains in service. Before beginning the combustion, the absorption apparatus is attached to the tube which is then burned out in a current of oxygen for thirty minutes. At the end of this time the absorption tubes are weighed and again attached to the tube.

The right end of the tube up to two burners to the left of Section D is then allowed to cool to the temperature of the room. At this moment the boat is inserted into the tube. As soon as the boat is in position, the burners are lighted under the right end of Section G and under Section D. It is recommended to cover the furnace over Section D with asbestos board or with brick, to insure maximum heat of the catalyst. As soon as the requisite temperature of the catalyst is developed, it turns light yellow in color. At this stage the burners are lighted under the right end of the furnace close to the right end of Section E. Following that moment the flames are regulated approximately in the same manner as in the Dennstedt combustion. One has to adapt himself to the nature of the substance.

The combustion is terminated when all the carbon in the boat or on the tube in the region of the boat is burned off. On burning



usual substances the combustion lasts about 10–15 minutes from the moment when the catalyst reaches the requisite temperature until the end of the operation.

After the combustion is completed the flames corresponding to Section D and to the right of it are turned off and oxygen is passed for 20 minutes. The absorption tubes are weighed immediately. Generally a combustion consumed 45 minutes, including the two weighings.

## THE STRUCTURE OF YEAST NUCLEIC ACID.

### III. AMMONIA HYDROLYSIS.

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, January 31, 1918.)

Since the tetranucleotide structure of yeast nucleic acid has been generally accepted, the efforts of workers in this field of investigation have been devoted to a search for an explanation of the mode of linkage between individual nucleotides. The theory of the structure of yeast nucleic acid was established through the discovery of methods which yielded on the one hand the individual nucleotides and on the other the pyrimidine nucleotides. For the explanation of the further details in the structure of the substance it was necessary to obtain a fragment of the nucleic acid molecule that would possess a more complex structure than the simple mononucleotides. Attempts in this direction were made by Thannhauser and Dorf-müller<sup>1</sup> and by Walter Jones<sup>2, 3, 4</sup> and his coworkers. Thannhauser and Dorfmüller reported the discovery of a trinucleotide, Walter Jones with his coworkers the discovery of two dinucleotides. The conclusions of Thannhauser and Dorfmüller were criticised by Jones who very convincingly exposed the weak points in the arguments of Thannhauser.

However, it is now found that the claim of Jones and Germann and Jones and Read to have isolated an adenine-uracil dinucleotide was not well founded. Following exactly the same conditions of analysis as given by these authors a brucine salt was obtained which possessed analytical values required by the dinucleotide. On recrystallization out of 35 per cent ethyl alcohol this brucine salt was separated into two fractions, one analyzing for uridinephosphoric

<sup>1</sup> Thannhauser, S. J., and Dorfmüller, G., *Z. physiol. Chem.*, 1915, xcv, 259.

<sup>2</sup> Jones, W., and Richards, A. E., *J. Biol. Chem.*, 1914, xvii, 71.

<sup>3</sup> Jones, W., and Germann, H. C., *J. Biol. Chem.*, 1916, xxv, 93.

<sup>4</sup> Jones, W., and Read, B. E., *J. Biol. Chem.*, 1917, xxix, 123; xxxi, 39.

acid, the other for adenosinephosphoric acid. Out of 120 gm. of the mixed salt there were obtained 50 gm. of the first portion and 70.0 gm. of the second. The first salt was converted into a barium salt which possessed the crystal form, the optical rotation, and the analytical values of the recently described<sup>5</sup> barium salt of uridinephosphoric acid.

The second fraction was also converted into a barium salt which separated out of a concentrated aqueous solution. However, it was impossible thus far to obtain the salt in crystalline form. Work in this direction is in progress.

It is evident on the basis of this experience that as far as the yeast nucleic acid is concerned the largest fragment obtained up to the present is a mononucleotide. Hence the problem of the mode of linkage between the individual nucleotides still awaits its solution. On the other hand the work done in Jones' laboratory and our recent work have advanced further proof for the tetranucleotide structure of yeast nucleic acid, since on cleavage of the nucleic acid it is now possible to isolate the following three mononucleotides in pure form: guanylic acid, uridinephosphoric acid, and cytidinephosphoric acid; and there is reasonable hope that adenosinephosphoric acid also will be prepared in pure form.

#### EXPERIMENTAL.

Crude nucleic acid in lots of 100.0 gm. each in 500 cc. water and 50 cc. of 25 per cent ammonia water were hydrolyzed for 1 hour in an autoclave at 115°C. The reaction product was filtered and to the filtrate an equal volume of 98 per cent alcohol was added. A precipitate was thus formed which was removed by filtration. The filtrate was concentrated to a third of the original volume under diminished pressure (between 12 and 15 mm.), the temperature of the water bath not exceeding 40°C. To the concentrated solution was added again an equal volume of 98 per cent alcohol. To the filtrate a 25 per cent solution of basic lead acetate was added as long as a precipitate formed.

<sup>5</sup> Levene, P. A., *J. Biol. Chem.*, 1918, xxxiii, 229.

The lead precipitate was ground up in a solution containing 5 per cent lead acetate and filtered. The operation was repeated three times. The washed lead precipitate was suspended in water containing barium hydroxide and decomposed by means of hydrogen sulfide. Care was taken to keep the solution slightly on the alkaline side. The excess of hydrogen sulfide was removed by aeration; the solution was then made distinctly alkaline, filtered, again neutralized, and concentrated to a volume of 500 cc. The barium was then removed quantitatively and from the solution the brucine salts were prepared in the usual way.

The analysis of the mixed brucine salts gave the following value.

0.200 gm. substance gave 15.4 cc. N at 18°C. and 747.2 mm.

For cytosine-uracil dinucleotide  $C_{19}H_{25}N_7P_2O_{15} \cdot 4(C_{23}H_{26}N_2O_4) + 14 H_2O$ .

	Calculated:	Found:
N.....	8.46	8.88

This material was recrystallized nine times. The final product gave the following analytical results.

0.200 gm. substance gave 12.4 cc. N at 17°C. and 757.2 mm.

0.1012 " " " 0.1970 gm. CO<sub>2</sub>, 0.0582 gm. H<sub>2</sub>O, and 0.0058 gm. ash.

	Calculated for $C_{26}H_{33}N_6PO_7 + 7H_2O$ :	Found:
C.....	53.20	53.08
H.....	6.51	6.43
N.....	6.80	7.26

This brucine salt was then converted into the barium salt. The salt crystallized in the manner described in the previous communication. It was redissolved in a small volume of a 10 per cent solution of sulfuric acid. The solution was neutralized with barium hydroxide, filtered, and allowed to crystallize at room temperature. The figure illustrates the crystals.

The substance gave the following analytical data.

0.200 gm. substance required for neutralization 8.92 cc. of 0.1 N acid.

0.1052 " " " gave 0.0912 gm. CO<sub>2</sub>, 0.0230 gm. H<sub>2</sub>O, and 0.0514 gm. ash.

	Calculated for $C_8H_{11}N_5O_8P_2Ba$ :	Found:
C.....	23.50	23.64
H.....	2.41	2.45
N.....	6.10	6.24
$Ba_2P_2O_7$ .....	48.97	48.86

The optical rotation of the substance was the following:

$$[\alpha]_D^{20} = \frac{+0.14^\circ \times 100}{1 \times 4} = +3.5^\circ.$$

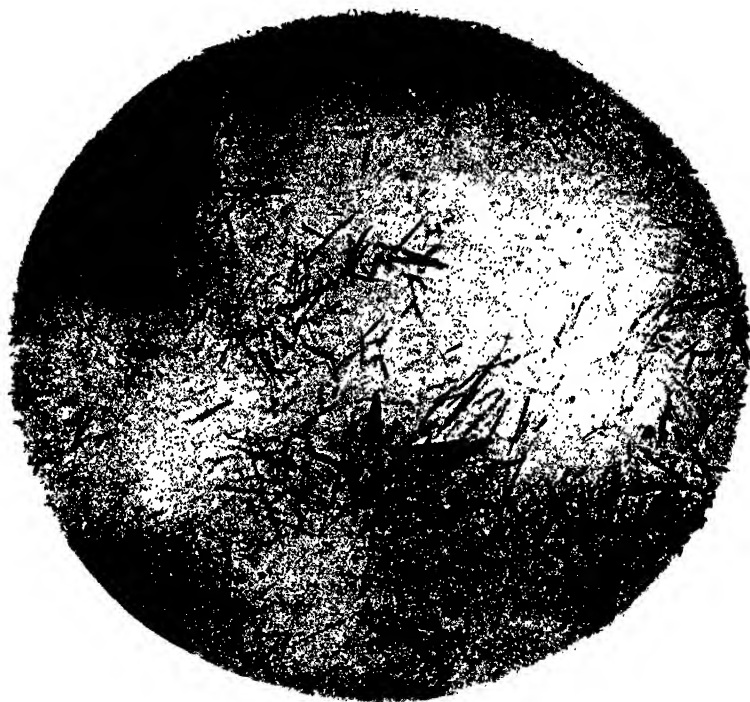


FIG. 1. Barium salt of uridinephosphoric acid.

## SYNTHESIS AND OXIDATION OF TERTIARY HYDROCARBONS.

BY P. A. LEVENE AND L. H. CRETCHER, JR.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, January 31, 1918.)

The present investigation was evolved from the previous work on the structure of branched chain fatty acids. Levene and Allen<sup>1</sup> have called attention to the lack of a convenient method for determining the location of the tertiary carbon atom in the branched chain fatty acids. An attempt was made to solve the problem by the oxidation of the fatty acids themselves in the hope that the tertiary carbon atom, as the one most susceptible to oxidation, might prove to be the point of disruption of the carbon chain. It is still possible that the solution of the problem may be reached by this mode of attack. Experience has shown, however, that the oxidation of any fatty acid takes place at more than one point of its carbon chain. Because of this the products of oxidation are numerous and it is not always an easy task to formulate the structure of the original molecule on the basis of many fragments. Hence it was thought that an advantage might be gained if prior to oxidation the molecule of the acid could be so transformed as to possess fewer points susceptible to the action of oxidizing agents. It was natural to think in this connection of the hydrocarbons, since every fatty acid is readily convertible into the corresponding hydrocarbon.

Can tertiary hydrocarbons be oxidized by a permanganate solution, or by some other oxidizing agent? The literature on the subject is very meager. In 1901, Zelinsky and Zelikow<sup>2</sup> made the observation that 3-methylpentane is readily oxidized by means of potassium permanganate. Prior to that observation it was universally accepted that saturated aliphatic hydrocarbons are not attacked by this reagent.

<sup>1</sup> Levene, P. A., and Allen, C. H., *J. Biol. Chem.*, 1916, xxvii, 433.

<sup>2</sup> Zelinsky, N., and Zelikow, J., *Ber. chem. Ges.*, 1901, xxxiv, 2865.

The observation of the Russian chemists has not been followed up by a study of the products of oxidation, nor has it been extended to observations on other hydrocarbons. The present work was undertaken with the object of obtaining the lacking information.

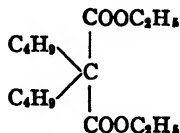
Since few tertiary hydrocarbons are readily accessible the work naturally fell into two parts, one directed towards the synthesis of tertiary aliphatic hydrocarbons and the other to the study of their behavior towards potassium permanganate. The work is reported in its present incompleteness because one of the authors has accepted a commission with the United States Army.

The most practical and economical way for the preparation of the hydrocarbons was found to be the one based on the reduction of acids obtained by the malonic ester synthesis. In a general way the routine adopted in the work of Levene and Allen was also followed here. However, a marked improvement was introduced in the method of the reduction of esters to the corresponding alcohols. The details are given in the experimental part. Up to the present there were prepared all the intermediate substances leading up to 2-butylhexane as well as the hydrocarbon, also all the intermediate products leading up to 4-butyloctane but not the hydrocarbon.

Regarding the behavior of 2-butylhexane towards permanganate it was found that it readily underwent oxidation in an alkaline solution of the reagent. However for the purpose of the study of the products of oxidation, special conditions had to be chosen. Namely, it was found that when the oxidation was permitted to proceed at moderately elevated temperatures (between 80–90°C.), the only oxidation products that could be detected were formic and carbonic acids. On the other hand when the oxidation was conducted at a temperature in the neighborhood of 25°C. evidence was obtained of the formation of butyric acid. This acid was identified as its silver salt. The present experiment was carried out only on a small sample of material. It is intended to continue the work on a larger scale.

The experience gained up to the present is important in as far as it indicates the conditions of experimentation which will permit the isolation of intermediate products of oxidation of the tertiary hydrocarbons.

## EXPERIMENTAL.

*Diethyl Dibutylmalonate.*

This ester was obtained by the action of butyl iodide and sodium ethylate upon diethyl malonate. One molecule of sodium ethylate and one of butyl iodide were added to malonic ester and the mixture was boiled until neutral. After the first substitution was complete another molecule of sodium ethylate and butyl iodide were added and the mixture was again boiled on the water bath until it was no longer alkaline to litmus. The attempt to make both substitutions at the same time, *i.e.* by heating the malonic ester with two molecules of sodium ethylate and of butyl iodide, gave unsatisfactory results. Diethyl dibutylmalonate boils at 153–154° at 14 mm. (corrected).

0.1006 gm. substance gave 0.2464 gm. CO<sub>2</sub> and 0.0938 gm. H<sub>2</sub>O.

	Calculated for C <sub>18</sub> H <sub>30</sub> O <sub>6</sub> :	Found:
C.....	66.58	66.80
H.....	10.29	10.42

*Dibutylmalonic Acid.*

This substance was prepared by the saponification of the corresponding ester in the following manner. Malonic ester was converted into diethyl dibutylmalonate as described above. For the preparation of this acid in quantity the ester was not isolated, but to the alcoholic solution of the ester resulting from the treatment of malonic ester with sodium and butyl iodide there was added a solution of potassium hydroxide in the minimal amount of water. The solution was boiled on the water bath under a reflux for 10 hours to complete the saponification, the mixture was then transferred to a large beaker, the alcohol evaporated, a small amount of water was added, and the acid liberated by the addition of concen-



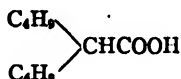
trated hydrochloric acid to the cooled solution. The acid separated in solid form. It may be recrystallized from benzene from which solvent it separates in long, transparent, prismatic needles. It is slightly soluble in water but practically insoluble in concentrated salt solution.

114 gm. of this dibasic acid were obtained from 100 gm. of malonic ester, corresponding to a yield of 84 per cent of the theory. Dibutylmalonic acid melts at 163° with slight decomposition.

0.1461 gm. substance gave 0.3260 gm. CO<sub>2</sub> and 0.1199 gm. H<sub>2</sub>O.

	Calculated for C <sub>11</sub> H <sub>22</sub> O <sub>4</sub> :	Found:
C.....	61.15	60.85
H.....	9.20	9.19

### *2-Butylhexylic Acid.*



The following will describe a typical experiment by which this acid was prepared. 120 gm. of dibutylmalonic acid were heated in a distilling flask to 180°. This temperature was maintained until the carbon dioxide was no longer evolved. The liquid remaining in the flask was then distilled. At atmospheric pressure 2-butylhexylic acid boils at 255° (corrected); at 16 mm. the boiling point is 153°. The specific gravity at 16° is 0.899. The yield was 90 gm

0.1681 gm. substance gave 0.4285 gm. CO<sub>2</sub> and 0.1711 gm. H<sub>2</sub>O.

	Calculated for C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> :	Found:
C.....	69.76	69.51
H.....	11.62	11.39

### *Ethyl 2-Butylhexylylate.*

130 gm. of the corresponding acid were boiled for 8 hours with four molecules of absolute alcohol and a small amount of sulfuric acid. The yield of ester was 144 gm. Ethyl 2-butylhexylylate boils at 114–115° at 15 mm. (corrected).

0.1004 gm. substance gave 0.2652 gm.  $\text{CO}_2$  and 0.1060 gm.  $\text{H}_2\text{O}$ .

	Calculated for $\text{C}_{12}\text{H}_{24}\text{O}_2$ :	Found:
C.....	72.00	71.98
H.....	12.00	11.85

### *2-Butylhexyl Alcohol.*

The method used for the reduction of ethyl 2-butylhexylate to 2-butylhexyl alcohol was essentially the same as that described by Levene and Allen. However, it has been found, in this instance, that the time of reduction can be reduced to half providing the following two changes in procedure are observed: The mixture of alcohol and ester should be added so slowly that no initial cooling is required. That stage of the reaction having been reached when the addition of alcohol ceases to cause more refluxing, the remainder of the alcohol necessary to affect complete solution of the sodium is rapidly added. In this way the reduction may be completed in about 25 minutes and the yield in no way impaired. The yield of alcohol corresponds to between 65 and 70 per cent of that theoretically possible. The acid resulting from saponification was recovered after the manner described by Levene and Allen. The alcohol boils at  $218\text{--}219^\circ$  (corrected). Its specific gravity is 0.836.

0.1447 gm. substance gave 0.4044 gm.  $\text{CO}_2$  and 0.1802 gm.  $\text{H}_2\text{O}$ .

	Calculated for $\text{C}_{10}\text{H}_{22}\text{O}$ :	Found:
C.....	75.95	76.14
H.....	13.92	13.93

### *2-Butylhexyl Iodide.*

2-Butylhexyl alcohol was boiled for 5 hours with three molecules of constant boiling hydriodic acid. The yield of iodide was about 80 per cent. The boiling point is  $124\text{--}125^\circ$  at 13 mm. (corrected). The specific gravity is 1.267.

0.2242 gm. substance gave 0.1956 gm.  $\text{AgI}$ .

Calculated for $\text{C}_{10}\text{H}_{21}\text{I}$ :	Found:
47.38	47.16

*2-Butylhexane.*

2-Butylhexane was prepared by the reduction of the corresponding iodide with zinc and glacial acetic acid. 75 gm. of iodide were mixed with 350 cc. of glacial acetic acid and allowed to stand 3 days on the water bath under a reflux. 60 gm. of powdered zinc were added in small amounts. On addition of water two layers were formed; the upper layer of hydrocarbon, after refractionation, weighed 25 gm. The boiling point of 2-butylhexane is  $165^{\circ}$  (corrected). The specific gravity is 0.738.

0.1010 gm. substance gave 0.3726 gm.  $\text{CO}_2$  and 0.1394 gm.  $\text{H}_2\text{O}$ .

	Calculated for $\text{C}_{10}\text{H}_{22}$ :	Found:
C.....	84.51	84.41
H.....	15.49	15.41

*Diethyl 2-Butylhexylmalonate.*

90 gm. of malonic ester (an excess of 20 per cent above the theory) were converted into the monosodium derivative by the addition of 10.6 gm. of sodium in alcohol; 125 gm. of 2-butylhexyl iodide were slowly added and the mixture was boiled on the water bath until neutral. The yield of the substituted ester was 90 gm. This ester boils at  $180^{\circ}$  at 14 mm. (corrected).

0.1064 gm. substance gave 0.2664 gm.  $\text{CO}_2$  and 0.0972 gm.  $\text{H}_2\text{O}$ .

	Calculated for $\text{C}_{27}\text{H}_{50}\text{O}_4$ :	Found:
C.....	68.00	68.29
H.....	10.60	10.23

*2-Butylhexylmalonic Acid.*

This acid was prepared from the corresponding ester by saponification with potassium hydroxide in alcohol solution. It crystallizes from low boiling petroleum ether in transparent, rhombic needles which melt at  $88^{\circ}$  (corrected).

0.1018 gm. substance gave 0.2384 gm.  $\text{CO}_2$  and 0.0876 gm.  $\text{H}_2\text{O}$ .

	Calculated for $\text{C}_{21}\text{H}_{40}\text{O}_4$ :	Found:
C.....	63.93	63.87
H.....	9.83	9.63

*4-Butyloctylic Acid.*

The method used for the preparation of this acid was exactly analogous to that described for the preparation of 2-butyloctylic acid. The boiling point is 173–174° at 12 mm. (corrected). The specific gravity is 0.901.

0.1034 gm. substance gave 0.2736 gm. CO<sub>2</sub> and 0.1108 gm. H<sub>2</sub>O.

	Calculated for C <sub>12</sub> H <sub>22</sub> O <sub>2</sub> :	Found:
C.....	72.00	72.16
H.....	12.00	12.00

*Ethyl 4-Butyloctylate.*

In the preparation of this substance 36 gm. of 4-butyloctylic acid were boiled for 6 hours with four molecules of absolute alcohol and a few drops of sulfuric acid. The refractionated product weighed 32 gm. It boils at 139° at 10 mm. (corrected).

0.1000 gm. substance gave 0.2692 gm. CO<sub>2</sub> and 0.1104 gm. H<sub>2</sub>O.

	Calculated for C <sub>14</sub> H <sub>26</sub> O <sub>2</sub> :	Found:
C.....	73.67	73.41
H.....	12.24	12.35

*4-Butyloctyl Alcohol.*

28 gm. of the above ester upon reduction yielded 14 gm. of alcohol. The boiling point is 139° at 15 mm. Its specific gravity is 0.841.

0.1266 gm. substance gave 0.3584 gm. CO<sub>2</sub> and 0.1266 gm. H<sub>2</sub>O.

	Calculated for C <sub>12</sub> H <sub>22</sub> O:	Found:
C.....	77.29	77.20
H.....	13.57	13.90

*4-Butyloctyl Iodide.*

This iodide was prepared by boiling a mixture of 10 gm. of the corresponding alcohol with four molecules of constant boiling hydriodic acid. The yield of iodide was 14 gm. It boils at 143° at 8 mm. Specific gravity, 1.194.

0.1992 gm. substance gave 0.1566 gm. AgI.

	Calculated for $C_{12}H_{24}I$ :	Found:
I.....	42.90	42.32

### *Oxidation of 2-Butylhexane.*

The hydrocarbon was oxidized by allowing it to stand in contact with an alkaline solution of potassium permanganate at room temperature for about 4 weeks. Three equivalents for permanganate were used, enough water being added to make the concentration of the permanganate 5 per cent at the beginning of the oxidation. Only a small amount of the hydrocarbon was oxidized. The unoxidized portion was separated from the aqueous layer, the latter acidified and distilled with steam into diluted ammonia. This distillate was concentrated to a small volume *in vacuo* and the fatty acid precipitated as the silver salt.

0.0971 gm. silver salt gave on ignition 0.0531 gm. silver.

	Calculated for $C_{12}H_{24}O_2Ag$ :	Found:
Ag.....	55.38	54.68

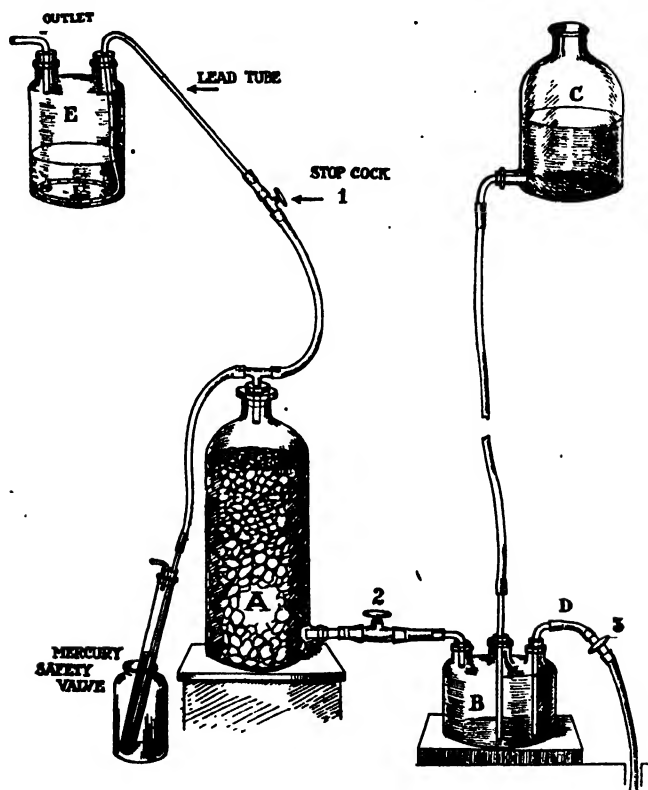
## A HYDROGEN SULFIDE GENERATOR.

By LOUIS SATTLER.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received, January 24, 1918.)

A great variety of hydrogen sulfide generators have been described. However, it still remained a problem to find one which would satisfy



the needs of a laboratory where the gas is used continually in a comparatively large volume. The apparatus here described has given satisfactory service in this laboratory. The construction is apparent from the sketch.

By shortening or lengthening the tube connecting the reservoir *C* and the mixing bottle *B* the gas is delivered at any desired pressure. Furthermore, the capacity of the generator can be readily altered to hold either larger quantities of iron sulfide, *A*, or larger quantities of acid, *C*.

After the aspirator bottle *A* has been filled with iron sulfide, diluted hydrochloric acid (about 50 per cent by volume) is poured into a reservoir bottle *C*. By opening stopcocks 1 and 2 the acid is allowed to flow into the mixing bottle *B*. This bottle should be seven-eighths filled. Then stopcock 1 is closed and enough acid poured into *C* so that when stopcock 1 is again opened there are about 3 in. of acid left in the reservoir bottle *C* after *B* is filled.

The waste acid is removed by closing stopcock 2 and opening stopcock 3. The pressure from the reservoir starts the syphon *D*. A Woulff bottle, *E*, is used for washing the gas. This is partly filled with water into which leads a submerged lead coil sealed at the end and perforated with small holes. Any excessive gas pressure is taken care of by a safety device consisting of a glass tube which may be lowered to any depth into mercury.

The generator in this laboratory holds 50 lbs. of iron sulfide and 14 liters of acid. The cost of material was twenty-seven dollars.

## THE CHEMICAL BASIS OF AXIAL POLARITY IN REGENERATION.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

### I.

When a piece of a stem is cut out from a plant one or more new shoots will usually arise at the apical, and roots at the basal end of the piece. This phenomenon of axial polarity was explained by the older botanists as being due to a flow of shoot-forming substances to the apex and of root-forming substances to the base. The gathering of these substances at opposite ends of the piece was believed to be responsible for the phenomenon of polarity in regeneration. While this may or may not be correct, the writer has recently found facts which suggest an additional or a different mechanism for this polarity, namely, that the apical bud suppresses the growth of the buds situated more basally in the stem by sending out inhibitory substances in a basal direction.

The experiments were made on *Bryophyllum calycinum*. Each node of the stem of this plant has two leaves in an opposite position, and in the axil of each leaf is found a dormant bud capable of giving rise to a shoot. The line connecting two buds of one node is at right angles to the line connecting the two buds of the next node.

*Experiment I.*—A piece of stem, containing six or more nodes, is cut out from a plant, all the leaves are removed and the piece is put into a horizontal position with the line connecting the two buds of the most apical node vertical. In this case both buds in the apical node may begin to grow, but as a rule only the upper bud will continue to grow, while the growth of the lower bud will soon stop altogether or will be considerably retarded. None of the buds in the other nodes will grow out. Roots will grow chiefly on the under side of the stem, but in the last node and at the cut end they may form on the upper side as well as on the lower side of the stem.



*Experiment II.*—The same as Experiment I., except that the upper apical bud is cut out. In this case the lower apical bud will grow rapidly, but in addition one or both of the buds of the node next to the apical will grow out. These buds never grow out when the upper apical bud is preserved and healthy.

*Experiment III.*—The same as the previous experiment except that the lower apical bud is removed, while the upper one is preserved. In this case, the upper apical bud will grow out, but none of the others.

It follows from these experiments that the upper apical bud inhibits or retards the growth of the lower apical bud as well as that of the rest of the buds; while the lower apical bud can not suppress the growth of the buds in the node behind. The writer has repeated these experiments in many modifications, among which those on longitudinally split stems are the most striking. The results were uniform.

All these observations are intelligible if we assume that a bud when it begins to grow produces and sends out inhibitory substances toward the base of the stem. These substances flow in the conducting vessels in the same half of the stem where the bud lies; when one apical bud is above and one below, the two buds in the next node are in a lateral position between the upper and lower half of the stem. Hence the inhibitory substances sent out by the upper apical bud can reach the two buds in the next node behind and inhibit their growth, since these buds lie directly below or on the lower level of the conducting vessels from the upper apical bud; while inhibitory substances sent out by the lower apical bud can not reach the buds in the node behind in large quantity, since these buds are on the upper level or slightly above these conducting vessels. When the two lateral buds grow out they will inhibit the growth of all the buds behind, each bud covering a territory of one half stem.

The alternative hypothesis assumes that since the apical bud is the first to grow out it will absorb all the shoot-forming material.<sup>1</sup>

<sup>1</sup> This form of inhibition exists apparently in the leaf where the shoots which grow out first prevent other notches in the leaf from giving rise to shoots by absorbing the material needed for shoot formation. SCIENCE, 1917, XLV., 436; XLVI., 115; *Bot. Gaz.*, in print.

If we assume that the shoot-forming material has a tendency to rise this hypothesis may explain the facts also. But the following experiment, which seems crucial, decides in favor of the other assumption.

A piece of stem containing a number of nodes is suspended horizontally, as in the previous experiments, with the two apical buds in a vertical line. All the leaves are removed with the exception of those at the apical node. Here the petioles of the leaves are left attached to the stem, the leaves having been cut off. The petioles will wilt in a week or ten days, but until then will prevent or retard the growth of the apical buds in their axils. The buds in the next node will begin to grow out and as soon as the petioles have fallen off the apical buds will also begin to grow.

The next step is decisive for testing the two hypotheses. If the inhibiting effect of the apical buds on the more basal buds is due to the fact that the buds which grow out first attract all the material from the basal part of the stem, the buds in the node behind the apical one, which grew out first, should continue to outstrip in growth the apical buds which began to grow out later. But if the inhibiting effect is due to an inhibitory substance being sent in the direction toward the base by the growing bud, the most apical bud should soon outstrip in growth those situated in the next node behind, although the latter had an earlier start. For according to this theory, the most apical buds should be sending substances toward the base which inhibit the growth in the next bud; while the most apical buds receive no such inhibitory substances. The results of the experiment are quite clear. As soon as the petioles at the apex fall off the axillary buds at the apex begin to grow out and soon not only outstrip in size those of the next buds behind but actually retard or stop the growth of the latter. This phenomenon seems intelligible only on the assumption that a growing bud sends out substances toward the base of the stem which directly inhibit the growth of the other buds.

## II.

If the inhibition of shoot formation is due to special inhibitory substances it should be possible to show that the inhibition varies quan-

titatively with the mass of inhibitory substances produced in the growing bud, or with the mass of the latter. While the bud is too small for convenient quantitative experimentation, it can be carried out satisfactorily with the leaf. In a former paper the writer had shown that the leaf of *Bryophyllum* sends out material toward the base of the stem which favors root formation; and it also seemed possible that the leaf might send out substances in a basal direction which inhibit shoot formation. The sap from the leaf flows in conducting vessels situated in the same half of the stem where the leaf is attached.

When we suspend a stem of *Bryophyllum* with six or more nodes horizontally, and remove all the leaves except the two in the apical node, the stem will form no shoots as long as the leaves are alive, but an abundance of roots is produced in the stem. The two leaves, therefore, inhibit all the shoot formation in the buds situated basally from the leaf. When we remove one of the two apical leaves the axillary bud of this leaf will grow out and it will have the same inhibiting effect as the leaf in the previous experiment. We now make the following experiment.

Twelve long stems from which all leaves except one of the two apical ones have been removed are suspended horizontally, and the free axillary bud opposite the leaf is also cut out. Six stems are suspended with the leaf above, six with the leaf below. There is a striking difference in the two sets. When the leaf is below, shoots will develop either in the two lateral buds of the first node behind the leaf, or on the upper side of the second node behind the leaf. When the leaf is above, no shoots will develop in the next node behind the leaf but one shoot may grow in the second node behind the leaf, *on the lower side alone*. These shoots will develop more slowly than those in the stems whose leaf is on the lower side.

This is exactly the result which we should expect if the leaf sends out substances inhibiting shoot formation toward the base of the stem. These substances, being identical with or accompanying the root-forming substances, flow on that side of the stem where the leaf is, but have naturally a tendency to flow downward and not to flow upward. Hence, when the leaf is below it is possible for shoots to form in some (about 50 per cent.) of the stems in the first node

behind the leaf, in which case the buds are on the upper level of the flowing sap; while when the leaf is above it is impossible for the buds in the first node behind the leaf to grow because they are on the lower level of the sap flow from the leaf. The bud on the lower side of the second node behind the leaf (when the latter is on the upper side of the stem) is outside the sap flow and hence it may develop.

When we work with a large apical leaf attached to a short stem (the free apical bud opposite the leaf is always removed in these experiments) containing only two nodes behind the leaf, everything is as described for long stems. When, however, the piece of stem behind the leaf is smaller, containing only one node, no shoot can grow on this stem even when the leaf is below. The mass of inhibitory substance sent out by a large leaf will flood the buds in this node with inhibiting material. Occasionally a bud starts to grow but stops before a leaflet has time to unfold. Such a stem will form an abundance of roots at the base. If, however, we reduce the size of the apical leaf by cutting away nine tenths of its mass, most or practically all the stems will form shoots in the node behind the leaf; but roots in such stems either do not develop at all or only with long delay.

The leaf, therefore, sends substances to the basal part of the stem which inhibit shoot formation and favor root formation, and the mass of these inhibitory substances decreases with the mass of the leaf, and apparently parallel with the mass of root-forming substances sent to the base of the stem.

Another experiment is equally instructive. We have seen that when long stems having all but one apical leaf removed (and the opposite free apical bud also removed) are suspended horizontally, with the leaf above, no shoot will form on the upper side of the stem. When we reduce the size of the leaf sufficiently this inhibition ceases.

Again the objection might be raised that the inhibiting effect of the leaf on shoot formation in the region behind the leaf is due not to an inhibitory substance being sent out by the leaf but by nutritive substances needed for the growth of shoots being sent into the leaf by the stem. This is highly improbable not only on the basis of our knowledge of these processes but also on account of the following fact. When we cut off a leaf without its petiole, leaving the

latter in connection with the stem, the petiole will dry out and fall off in a week or less. If, however, the petiole is detached from the stem but left attached to a leaf, it will not wilt, but remain fresh and green as long as the leaf is alive, which may be many months. This shows that nutritive material is furnished by the leaf to the stem, and not vice versa.

### III.

While these experiments show that the inhibiting influence of an apical bud on the growth of the more basal buds is due to one or more inhibitory substances being sent toward the basal end of the stem, the other main fact of polarity remains unexplained; namely, how it happens that the most apical bud grows out first. The writer is inclined to offer the following suggestion: In the normal plant, the substances inhibiting shoot formation are constantly flowing from the growing region toward the root of the plant. When we cut out a piece of stem and remove the leaves these substances will at first exist in every node, but will continue to flow toward the base. Hence the most apical node will be the first one to be free from these inhibitory substances and the bud or buds situated here can now begin to grow out. As soon as they grow out they will maintain a constant flow of inhibitory substances toward the base which will suppress the growth of buds in the more basal part of the stem.

The experiments, therefore, seem to prove that axial polarity in the regeneration of a stem is due to the fact that the apical bud (as well as an apical leaf) send out substances toward the base of a stem which inhibit the buds from growing out. These inhibitory substances may be identical with or may accompany the root-forming hormones. The most apical bud in an excised piece of stem will grow out first since it will be the first to be free from these inhibitory substances.

In a former paper the writer had pointed out that a leaf sends out substances, in an apical direction through the stem, which favor shoot formation.

## THE CHEMICAL MECHANISM OF REGENERATION.

By JACQUES LOEB.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

### I.

1.—It is known that if a plant or a lower animal is mutilated a new growth may take place by which the organism is once more rendered more or less complete, and this process has been termed regeneration. The regeneration of lost organs proceeds in plants usually from dormant buds near the wound, whose growth is called forth as a consequence of the mutilation. The phenomenon of regeneration has been interpreted in the purely verbal way by attributing to a plant or an animal some kind of feeling for its proper form (Noll's morphæstesia) or by the presence in the organism of a mystical agency, the "entelechy." Weismann pointed out that regeneration has a selective value since forms endowed with this power were more likely to survive. Others ascribed regeneration to a "stimulating" effect of the wound. These and similar attempts of explaining regeneration need not occupy our attention since they have not led to scientific results.

The earlier biologists, Bonnet and Duhamel, had a more chemical attitude towards the problem, assuming that a plant contained two kinds of sap, an ascending one which carried material for the shoot production, and a descending one carrying material for root production. If the apex is cut off in a plant, the ascending sap collecting near the cut will induce the growth of shoots from buds, and if the root is cut off, the collection of the descending sap at the base will induce there the growth of roots. Sachs accepted these views and generalized them by assuming as many different specific organ-forming substances in the plant (or animal) as there are organs. These specific substances were said to be present in minute quantities only. This assumption has received some support by recent observations on the effect of internal secretions, especially the thyroid gland.

In his early experiments on regeneration and heteromorphosis in animals the writer was able to point out that the chemical viewpoint of Bonnet, Duhamel and Sachs harmonized very well with his observations. Thus he had found that if a piece is cut from a stem of *Tubularia* (a hydroid) and suspended in sea water, a head (hydranth) will form at either end of the stem, but the head will form much more rapidly at the oral than at the aboral end of the piece; the difference may amount from a few days to a few weeks. Suppression of the head formation at the oral end (by withdrawal of oxygen at that end) accelerated the formation of the head at the aboral end, so that the latter often formed as early as the oral head in the pieces with normal oxygen supply. This is intelligible on the assumption that the head formation is determined by substances which normally flow towards the oral end of an excised piece of stem until a head has been formed, and that afterwards the direction of the flow is reversed; and that if the formation of a head at the oral end is suppressed by the withdrawal of oxygen at this end, the flow is reversed from the beginning and a head can develop at the aboral end as rapidly as the head at the oral end can under normal conditions. This conclusion could be submitted to a test by ligaturing an excised piece of stem of *Tubularia* anywhere between the two ends. This ligature should interrupt the flow of the hypothetical head-forming substances from the aboral towards the oral end, and hence should abolish the cause for the retardation of the head formation at the aboral end; and this was found to be true by Godlewski as well as by the writer (1).

While it was thus possible to show in this and in other cases that the observations on regeneration in animals agree with a chemical theory of regeneration, an adequate proof was lacking; since for this purpose quantitative determinations were required. The fact that the specific organ-forming substances of Sachs were unknown and the statement that they exist only in minute traces in an organism made it appear a hopeless task to undertake quantitative experiments. The writer has recently found that we can investigate the regeneration of the tropical plant *Bryophyllum calycinum* by quantitative methods with-

(1) J. LOEB, *La conception mécanique de la vie*, Paris, 1914 et *La dynamique des phénomènes de la vie*, Paris, 1908 (librairie Felix Alcan).

out having recourse to the hypothetical organ-forming substances of Sachs (1).

2.—The leaves of this plant possess the peculiarity of containing dormant buds in each of their notches (Fig. 2) which give rise to roots and shoots as soon as the leaf is separated from the plant, or about to fall off. An explanation of the mechanism of this "regeneration" must also explain the mechanism by which the regeneration is inhibited as long as the leaf is in connection with the whole plant. We shall try to show in this paper that regeneration or rather the growth of the dormant buds in the isolated leaf of *Bryophyllum* takes place as a consequence of the mass action of substances present, or formed in the leaf; and that this regeneration cannot take place as long as the leaf is part of the plant, because the substances needed for "regeneration" in the leaf flow into the stem of the plant. The writer has found a quantitative method by which this idea can be tested.

When a leaf of *Bryophyllum calycinum* is separated from its plant it produces new shoots, not in all of its notches, but in only a few; while when the leaf is cut into as many pieces as it contains notches, each notch will give rise to a new shoot. Hence we may ask the question, why do not all the notches grow out in an isolated leaf when it is not cut into pieces? It is obvious that here we are confronted with the problem of inhibition of regeneration in so simple a form that a definite solution can be found.

Each node of *Bryophyllum* gives rise to two leaves of equal size. The writer found that when two such sister leaves are isolated and kept under identical conditions they produce in equal time equal masses of shoots, as Table I indicates. Nine pairs of sister leaves were isolated and kept for 31 days on moist filter paper. The one set of nine leaves, weighing 12.022 gm., produced in that time 1.436 gm. of shoots; the nine sister leaves, weighing 11.861 gm., yielding 1.348 gm. of shoots.

Equal masses of sister leaves produce, therefore, approximately equal masses of shoots in the same time and under equal conditions. In this case the number of shoots in the two sets of sister leaves was not very different; we can easily cause a greater difference in the

(1) J. LOEB, *Science*, 1917, XLV, 436; XLVI, 115.



TABLE I.

Number of Leaves.	Weight of Leaves.	Shoots Produced by Leaves.		Mg. of Shoots Produced per Gram of Leaf.
		Number.	Weight.	
	<i>gm.</i>		<i>gm.</i>	
9	12.022	24	1.436	119
9	11.861	20	1.348	114

number by leaving one set of leaves intact and cutting their sister leaves into four pieces. Table II gives the record of such an experiment in which eight pairs of leaves were used; eight leaves were left intact while each of their sister leaves was cut into four pieces. Duration of experiment 35 days.

TABLE II.

	Weight of Leaves.	Shoots Produced by Leaves.		Mg. of Shoots Produced per Gram of Leaf.
		Number.	Weight.	
	<i>gm.</i>		<i>gm.</i>	
8 Leaves, intact.....	12.917	18	2.834	219
8 Leaves, each cut into 4 pieces.....	13.232	33	2.998	226

Although the leaves cut into four pieces produced almost twice as many shoots as their intact sister leaves, both produced approximately the same mass of shoots, namely 2.834 gm. and 2.998 gm. respectively. We may, therefore, state that equal masses of sister leaves produce the same mass of shoots even if the number of shoots differs considerably.

When the pieces into which a leaf is cut become too small, the notches may not regenerate, or, if they regenerate, they may do so with some delay. The mass of shoots produced by such leaves is, therefore, liable to be a little smaller than that produced by their intact sister leaves, though the difference is not great enough to obliterate the law. Table III gives a comparison between the masses of shoots produced in 12 intact leaves and their 12 sister leaves each of which was cut into 8 pieces. Duration of experiment 36 days.

TABLE III.

	Weight of Leaves.	Shoots Produced by Leaves.		Mg. of Shoots Produced per Gram of Leaf.
		Number.	Weight.	
	<i>gm.</i>		<i>gm.</i>	
12 Leaves, intact.....	20.200	31	4.755	230
12 Leaves, each cut into 8 pieces.....	20.135	94	3.982	200

The mass of shoots produced by the intact leaves is only slightly greater than that produced by their sister leaves, each of which was cut into 8 pieces.

3.—In all these experiments the masses of the two sets of sister leaves were approximately equal. It was necessary to find out how the mass of shoots would vary if the masses of the two sets of leaves varied considerably. For this purpose large pieces were cut out of the center of one set of leaves, while their sister leaves remained intact. It was found that the mass of shoots produced in the two sets of leaves varied approximately with the mass of the two sets of leaves. Table IV gives the results. The leaves dipped with their apices into water.

TABLE IV.

	Weight of Leaves.	Shoots Produced by Leaves.		Mg. of Shoots Produced per Gram of Leaf.
		Number.	Weight.	
	<i>gm.</i>		<i>gm.</i>	
1. (37 days):				
5 Leaves with center cut out.....	7.61	11	7.61	99
5 Sister leaves, intact.....	13.80	9	13.80	101
2. (25 days):				
7 Leaves with center cut out.....	9.899	21	1.213	123
7 Sister leaves, intact.....	16.935	25	1.995	118
3. (32 days):				
9 Leaves with center cut out.....	10.522	22	2.292	218
9 Sister leaves, intact.....	17.852	30	3.430	192

These experiments show that if we reduce the mass of one set of leaves by cutting out pieces in the center, while their sister leaves

remain intact, both sets will produce in equal time and under equal conditions shoots in proportion to their masses. This law explains the inhibition of shoot production in a leaf. Observation shows that, when we isolate a leaf of *Bryophyllum calycinum*, those notches will as a rule grow out first into shoots where the leaf is thickest; as soon as these grow out the rest of the notches are prevented from growing, inasmuch as all the material available in the leaf now flows into the notches where shoots first begin to grow out. This withdrawal of the material from the other notches determines the inhibition of growth in these notches, since the material available at any time for shoot production is, as we have seen, limited and fixed by the mass of the leaf. When we cut a leaf into as many pieces as there are notches, they may all regenerate since no other notch can withdraw the material available for the growth of each.

We have it entirely in our power to decide which notch in a newly isolated but intact leaf shall grow out first; all we need to do is to dip that notch into water. We do not know definitely how the increase in water supply can accelerate the growth of a notch, though we may guess that an acceleration of a hydrolytic process may be involved. By thus accelerating the rate of shoot production in a notch we suppress at the same time the growth of the other notches of the same leaf, inasmuch as the available material in the leaf will now flow into the growing shoot. How it happens that the material in the leaf flows to that notch or those notches which by chance first give rise to shoots can only be guessed for the present, and may, therefore, be omitted from the discussion.

The new shoots formed in the notches may be considered as parasites living on the leaf and finally consuming it completely. In all cells hydrolytic processes go on, until a definite chemical equilibrium is reached between hydrolytic and synthetic processes. The hydrolytic processes furnish the amino-acids, sugar, and other constituents needed for the growth of roots and shoots from the notches. In two sister leaves these hydrolytic processes will take place at the same rate as long as the conditions remain alike, and from this we can understand why in two sister leaves the production of shoots proceeds in proportion to the mass of the two leaves. It is probable that the assimilation in the leaf also contributes to the quantity of material

available for shoot production. This seems to follow from the fact that in the dark the rate of shoot production in a detached leaf is considerably diminished.

## II.

It has been observed by Wakker that when a piece of stem is left attached to a leaf of *Bryophyllum calycinum* the shoot production in the leaf may be inhibited (Fig. 1) and the observation has been confirmed by de Vries and by Goebel. Wakker attributed this inhibition to the "pressure" of the roots formed on the piece of stem, but it can be shown that the inhibition takes place also when the root production is prevented in such a piece of stem. The suppression of shoot formation in a leaf by a piece of stem attached to it should find its explanation on the same principle which was proved in the first part of this paper: namely, the withdrawal of the material needed for regeneration from the leaf by the stem. In order to test this idea we again need quantitative methods.

When we cut from a stem a piece containing one node, detach one of the two leaves, leaving the other leaf attached to the piece of stem (Figs. 1-2), and allow both leaves to dip with their apices into water, both leaves may form shoots but the mass of shoots produced by the two sister leaves is no longer equal. The leaf with a stem attached will produce a smaller quantity of shoots than the leaf without stem, and the difference in the mass of shoots produced by the two sister leaves can serve as a measure for the inhibiting effect of the piece of stem upon shoot production in the leaf. By this method it was found that if the masses of leaves are equal the inhibiting power of the stem increases with its size or mass. Table V may serve as an example.

Sixteen pairs of sister leaves were chosen, 16 leaves were attached each to a (half) (1) stem 1 cm. long; the 16 sister leaves had no stem attached. The latter produced in 17 days 55 shoots weighing 2.361 gm., the former 42 shoots weighing 1.677 gm. The inhibiting effect of the 1 cm. half stem upon shoot production in the leaf was, therefore,  $2.361 - 1.677 = 0.684$  gm. In a parallel experiment 14 pairs of sister leaves weighing almost exactly as much as the leaves in the first experiment were used; 14 leaves were attached each to a half stem 6

(1) It will become clear later on what is meant by half stem.

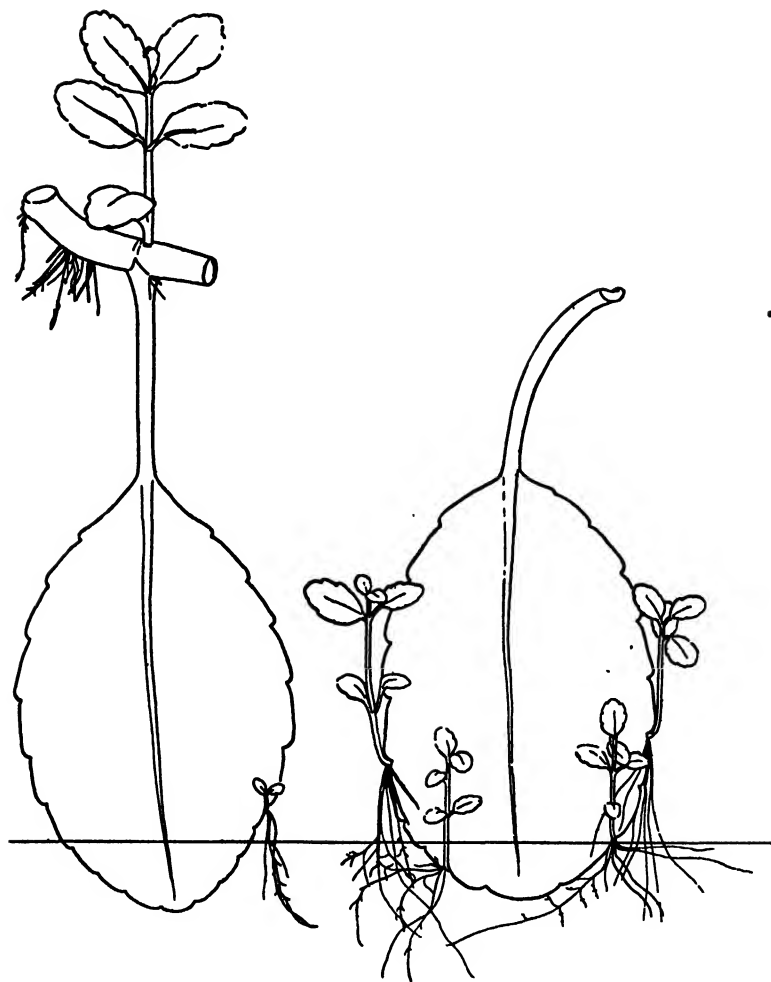


FIG. 1.

FIG. 2.

FIGS. 1 and 2.—Sister leaves dipping with their apices in water. Leaf in Fig. 2, without stem, has formed four shoots in four different notches. Leaf in Fig. 1, attached to a piece of stem, has just commenced to form one tiny shoot in one notch. The material which caused shoot production in leaf 2 was utilized by the stem in leaf 1 for the production of a large shoot on the stem, for callus formation at the basal end of the stem, and for a slight geotropic curvature. The drawing was made seven weeks after the beginning of the experiment.

cm. long, while the sister leaves had no stem attached. The latter produced in 17 days 49 shoots weighing 2.656 gm., while the leaves with stems produced only 16 shoots weighing 0.441 gm. The inhibiting effect of the 6 cm. stem upon shoot production in the leaves was, therefore,  $2.656 - 0.441 = 2.215$  gm., almost 4 times as much as that of the 1 cm. stem. The 1 cm. half stems weighed fresh 1.960 gm., the 6 cm. half stems 15.127 gm. *Hence the inhibiting power of the stems upon shoot production in the leaf increases with the length and mass of the stem but not as rapidly.*

Other experiments gave similar results.

The main problem before us was to decide the question whether or not the inhibiting effect of the stem upon shoot production in the leaf is due to the absorption by the stem of that material from the leaf which would have served for the shoot production in the latter, had

TABLE V.

Length of Stems.	Weight of Stems.	Weight of Leaves.	Weight of Shoots Produced in		Inhibiting Action of Stem.
			Leaves without Stems.	Leaves with Stems.	
cm.	gm.	gm.	gm.	gm.	gm.
1	1.960	40.5	2.361	1.677	0.684
6	15.127	43.0	2.656	0.441	2.215

it been detached from the stem. If this were the case it should be possible to show that the stem attached to a leaf increased in weight enough or more to account for the diminution of shoot production. In order to test this idea the following method was adopted. A piece from the stem of *Bryophyllum* containing one node with its two leaves, was cut out from a plant and the stem split longitudinally in the middle between the two leaves, leaving one half of the stem attached to each leaf. The half stem was removed from one leaf and weighed directly. The leaf whose half stem was cut off and the other leaf, with a half stem still attached to it, served for the experiment. After several weeks the stem was removed from the second leaf and weighed. It was invariably found that the stem had increased in weight to an amount exceeding the diminution in the production of shoots in the leaf attached to it.

While in the experiments mentioned in the first part of this paper, the fresh weights of the shoots were used for comparison, it was found necessary in this case to use the dry weights. It was found that, while the ratio of fresh and dry weight of shoots and leaves is fairly constant under the conditions of the experiments of the first part of this paper, this is no longer the case for the split stems. The percentage of dry weight in a split stem is smaller immediately after the stem is cut out of a plant than after it has been suspended in moist air for several weeks. Since the results of the experiment depend upon a comparison of the weight of a half stem when taken fresh from the plant and after some weeks, only the dry weights can give accurate results. In the following three sets of experiments, Tables VI and VII, the mass of each set of leaves was approximately the same, namely

TABLE VI.

Number of Experiment.	Length of Stem.	Dry Weight of Shoots Produced in		Difference (Inhibiting Effect of Stem on Shoot Production in Leaf).
		Leaves without Stems.	Leaves with Stems.	
	cm.	gm.	gm.	gm.
I	2	0.2156	0.0918	0.1238
II	4	0.2674	0.0558	0.2116
III	8	0.1662	0	≅0.1662

about 28 gm. fresh weight for 12 leaves. What interests us is, first, the difference in the dry weight of shoots produced in the leaves without and the leaves with half stems, second, the increase in the dry weight of the half stems left attached to the leaves during the experiment. According to our theory this latter increase should be equal to or greater than the difference in the shoot production in the two leaves. Each set of experiments was carried out with 12 pairs of sister leaves. In these experiments the leaf was always at the apex of the piece of stem, since it was found that the leaf sends its material into the basal part of the stem attached to it. The experiments lasted 29 days.

Table VII gives the increase in weight in the half stems during the experiment.

We see from Table VI that the 2 cm. stems reduced the shoot production in the leaves by 0.1238 gm. (dry weight). Table VII shows that the stems gained in the same time 0.2448 gm. in dry weight. Since this gain could have no other source than the supply of material from the leaf, the experiments make it probable that the suppression of shoot production in the leaf is caused by the absorption of material from the leaf by the stem. In experiment II the stems were twice as long as in experiment I and hence we notice that these stems reduced the shoot production in the leaf more than the shorter stems in experiment I, namely 0.2116 gm. dry weight. Hence we should expect to find that the larger stem in experiment II had also gained more in mass than the shorter stem in experiment I, and this was the case. The gain in mass of the stems was 0.3226 gm. dry weight.

TABLE VII.

Number of Experiment.	Length of Stem.	Dry Weight of Half Stem.		Increase in Dry Weight of Stem.
		At Beginning of Experiment.	At End of Experiment.	
	cm.	gm.	gm.	gm.
I	2	0.1592	0.4040	0.2448
II	4	0.3640	0.6866	0.3226
III	8	0.8290	1.1462	0.3172

In the third experiment the 8 cm. long stems suppressed the shoot production in the leaf completely, but the control leaves produced a considerably smaller mass of shoots (0.1662 gm. dry weight) than the control leaves in the two previous experiments, although the experiments were made simultaneously and at identical temperatures. It is possible that the illumination was not adequate or that the leaves were not in as good a condition as the others. The stems in this experiment gained also less than the shorter stems in experiment II, namely 0.3172 gm.

These and similar experiments show, first, that the inhibition of shoot production in a leaf by a piece of stem attached to it is accompanied by an increase in the mass of the stem, and, second, that this latter increase varies in the same sense as the inhibiting effect of the stem upon shoot production in the leaf. In view of this fact



and in view of the facts given in the first part of this paper it seems, therefore, highly probable that the inhibiting action of the stem upon the shoot production in the leaf is due to the absorption of material from the leaf by the stem.

It might be argued that our experiments would be more satisfactory if the increase in the amount of dry weight of the stem were exactly equal to its inhibiting effect upon the shoot production in the leaf instead of being in excess. Yet, the result we actually obtained is exactly the one which in the nature of the circumstances should be expected, since the leaf "naturally" sends its material into the stem and as a consequence the stem can draw from the leaf material more rapidly than can be done by notches of the leaf. When the notches begin to grow out new channels for the flow of sap to them will gradually have to be developed, while such conducting vessels or channels from leaf to stem are a part of the normal leaf. These ideas are supported by a number of observations. Thus it can be shown that the shoots which form in the free bud of a stem, as in Fig. 1, develop more quickly than the shoots in the notches of a leaf, even if the apex of the latter dips into water, thus indicating that the material of the leaf collects more rapidly in the stem than in the notches. Moreover, a piece of stem of a certain size will inhibit the shoot production in the notches of a leaf completely when the leaf is small, while the inhibition will be less complete when the leaf is larger. If the chances for the flow of material to the notches were the same as for the flow to the stem we should expect to find a constant coefficient of distribution of material between notches and stem which is not the case.

Finally, the stem not only inhibits shoot production in the leaf but also root production. If this is due to the absorption of material by the stem the latter should gain more than the equivalent of the inhibiting effect on shoot production alone.

### III.

The proof that the dry weight of a piece of stem left in connection with a leaf increases more than necessary to account for the inhibitory effect of the stem upon shoot production in the leaf, becomes of great significance when we investigate what becomes of this material in the

stem. We notice that it is utilized for at least three different forms of growth in the stem, namely, first, the formation of shoots from the dormant buds in the stem, second, the callus formation at the basal end of the stem, and, third, the geotropic curvature of the stem, all three of which may be observed in the same piece of stem attached to a leaf, as is the case in Fig. 1. When we cut a piece of stem with one leaf attached to it (as in Fig. 1) from a plant, such a stem will form a shoot from its free bud (in the axil of the leaf which was removed), and the mass of this shoot may equal or even slightly exceed the mass of shoots produced by the sister leaf which is not attached to a stem (as in Fig. 2). We can easily understand that the same material may be utilized for shoot production in the leaf and in the stem. But in addition such a stem produces a mass of callus at its basal end, the material for which also comes from the leaf. And finally, a third form of growth takes place in the stem at the expense of material from the leaf and this results in geotropic curvature.

The connection between geotropic curvature of a stem and the mass of the leaf connected with it can be demonstrated and since this relation is a recent discovery (as far as the writer is aware) it may be briefly discussed. The writer found a year ago that, if a piece of stem is cut out from *Bryophyllum* and suspended horizontally in moist air, the stem will gradually bend until it finally assumes the shape of a U with the concave side above (Fig. 3). It was found that this geotropic curvature is due to a longitudinal growth of a certain layer of cells in the cortex on the lower side of a horizontally placed stem; as a consequence of this growth the whole stem bends in the way described.

Now if our assumption is true that the inhibiting effect of the stem is due to the absorption of that material from the leaf which would have served for regeneration in the leaf; and that this material is utilized for the growth of certain cells in the stem, we should be able to show that the geotropic curvature increases with the mass of the leaf attached to the apical end of the stem. Our experiments show in a striking way that this is correct.

Stems, each of which having a leaf at the apical end were suspended horizontally in moist air; the leaves and stems were approximately equal in size. The leaves of one set were left intact while the mass of

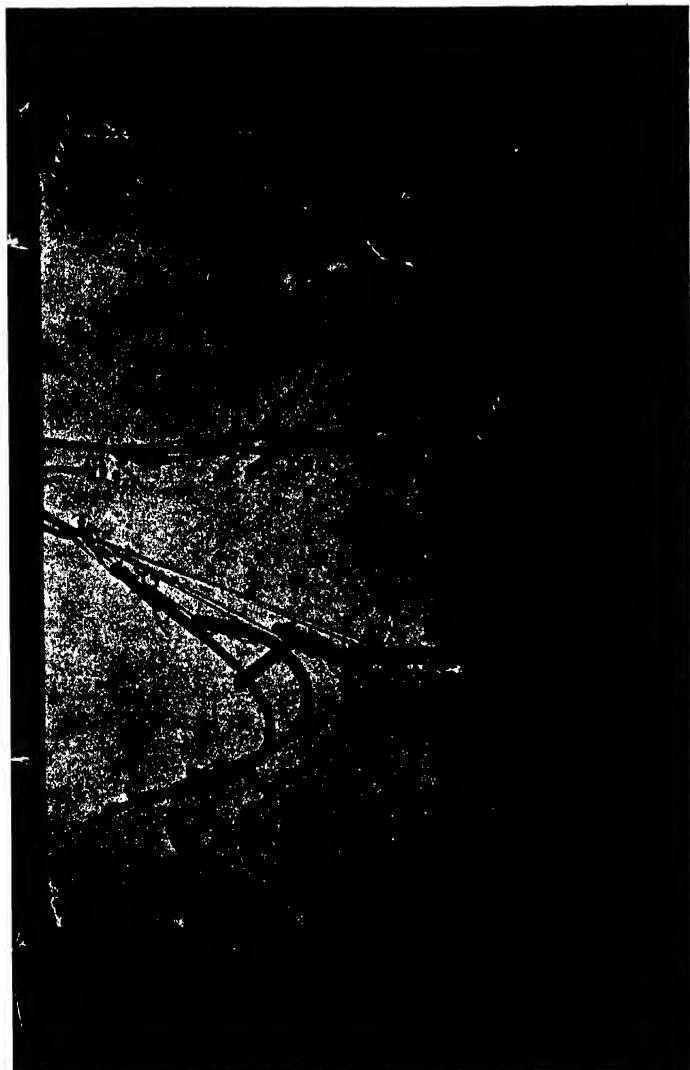


FIG. 3.—All the stems were originally straight and suspended horizontally in a glass case partly saturated with moisture. Each stem had one apical leaf. In the six stems at the right the leaf was reduced in size, while in the six stems at the left the size of the leaf was normal. The rate of geotropic curvature was considerably smaller in the stems at the right than in the stems to the left, showing that the rate of geotropic curvature increases with the mass of the apical leaf. The photograph was taken three weeks after the beginning of the experiment.

the leaves of the second set was reduced by cutting pieces out from the middle of the leaf. It was found that the rate of geotropic bending in the latter leaves was considerably slower than in the leaves with larger mass (Fig. 3). No matter in which way the mass of the leaf was reduced the rate of bending varied in the same sense as the mass of the leaf. Fig. 3 is a photographic reproduction of such an experiment. The leaves of the stems on the left side were intact, the mass of the leaves of the stems on the right side of the photograph was diminished by cutting off the front part of the leaves. Both sets of stems were straight at the beginning of the experiment and both sets were suspended simultaneously. The stems on the left, with the larger leaves, are bent more strongly than those with smaller leaves on the right. We have seen in the first part of this paper that the shoot production in a leaf varies with its mass. The geotropic curvature of a stem of *Bryophyllum* also varies with the mass of the apical leaf attached to it, no matter which part of the leaf is cut off. This supports the idea that the geotropic curvature of the stem occurs at the expense of that material which exists or is formed in the leaf and serves for the formation of shoots by the leaf when the latter is detached from the stem.

We can now understand why "regeneration" occurs when the leaf of *Bryophyllum* is separated from the plant and why it cannot occur as long as the leaf forms part of the whole organism.

The writer is under the impression that further quantitative experiments will put the problem of regeneration into the category of the phenomena of nutrition or mass action. Regeneration appears mysterious only as long as we do not perceive or measure the change in the distribution of the masses of the nutritive material, which must occur when a part is cut out from a whole organism.



## CHEMICAL BASIS OF CORRELATION.

### I. PRODUCTION OF EQUAL MASSES OF SHOOTS BY EQUAL MASSES OF SISTER LEAVES IN *BRYOPHYLLUM CALYGINUM*.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

In this paper the term correlation will signify the inhibiting influence which the growing buds of a leaf of *Bryophyllum calycinum* have upon the growth of other buds of the same leaf. It is generally known that in a complex organism the growth in one organ of the complex may inhibit the growth in other organs of the same complex.

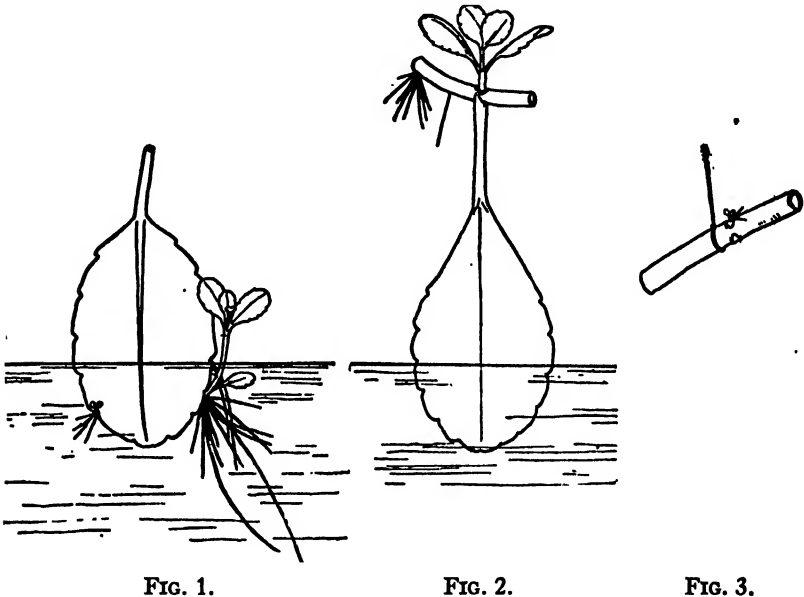
In former papers<sup>1</sup> the writer has shown that when in *Bryophyllum calycinum* one organ inhibits the growth of buds in another organ the inhibited organ contributes in some cases material to the growth in the inhibiting organ. It was known through the experiments of Wakker and DeVries<sup>2</sup> that if a piece of stem is left attached to a leaf of *Bryophyllum* the stem will inhibit the growth of the shoots in the notches of the leaf, while such shoots will grow if the leaf is entirely isolated from the stem. The writer was able to show that in such a case the leaf accelerates the growth of a shoot in the stem attached to the leaf. Thus figs. 1 and 2 are sister leaves, that is, leaves from the same node of a stem of *Bryophyllum*. Both are dipping with their tips in water.<sup>3</sup> Leaf 1, without a stem, has formed a shoot in 22 days, while the sister leaf in fig. 2 has formed no shoot, due to the inhibiting effect of the piece of stem attached to the leaf. The latter has accelerated the growth of the shoot in the piece of stem attached to the leaf, however, for a piece of stem of equal size without a leaf attached to it will in the same time form

<sup>1</sup> LOEB, J., BOT. GAZ. 60:249. 1915; 62:293. 1916; Science 41:704. 1915; The organism as a whole, p. 153. Putnam's Sons, New York. 1916.

<sup>2</sup> DEVRIES, H., Jahrb. Wiss. Bot. 22:35. 1890-91.

<sup>3</sup> The result is the same when the leaves are suspended in moist air instead of dipping into water.

no shoot or only a very tiny shoot (fig. 3). The inference was drawn that the inhibiting effect of the stem upon the leaf in fig. 2 was due to the fact that the leaf furnished the material required for the growth of shoots to the stem instead of to its own notches. This takes place even when no shoot is formed in the stem; in that case the material furnished by the leaf is stored in or consumed by certain



FIGS. 1-3.—Figs. 1, 2, sister leaves; leaf of fig. 2 still attached to stem, showing stem inhibits shoot formation in leaf; fig. 2 shows inhibition is accompanied by accelerating effect of leaf upon growth of shoot from stem, since in a piece of stem, suspended in moist air, as in fig. 3, production of shoots is suppressed or retarded.

cells of the stem, as indicated, for example, by callus formation and by geotropic curvature.<sup>4</sup>

The same principle was shown to hold if stems without leaves are suspended in moist air. In such cases the two buds of the most apical node of a long piece of stem grow out (fig. 4), and it can be shown that the basal part of the stem whose buds are inhibited from growing furnishes to the growing buds at the apex the material

<sup>4</sup>LOEB, J., Science 46: 547. 1917.

required for their growth, for if we cut out short pieces with one node only (fig. 4, *a*, *b*, *c*, *d*), the growth of the shoots from the buds is retarded. This is not the only factor of inhibition in this case, since the writer has recently shown<sup>5</sup> that a growing bud, as well as a leaf, seems to send out inhibitory substances toward the base of the stem which prevent the buds in the stem, situated more basally, from growing out. This factor of inhibition will not be considered in this paper.

We shall try to show in this paper that the quantity of material available for the formation of shoots is definite and limited, and that inhibition may result from the retention or utilization of part of this material by the inhibiting organ. A preliminary note of these results has already been published.<sup>6</sup>

Each notch of a leaf of *Bryophyllum calycinum* can give rise to a shoot when the leaf is cut off from the stem and suspended in moist air, but as a rule only a few of these notches will grow into new plants. When we cut the leaf into as many pieces as there are notches, practically each piece (very small ones only excepted) will give rise to a shoot. Figs. 5 and 6 are sister leaves. Leaf 5 is cut into as many pieces as there are notches, while leaf 6 is left intact. Both were kept on moist filter paper. Leaf 5 has given rise to a new shoot

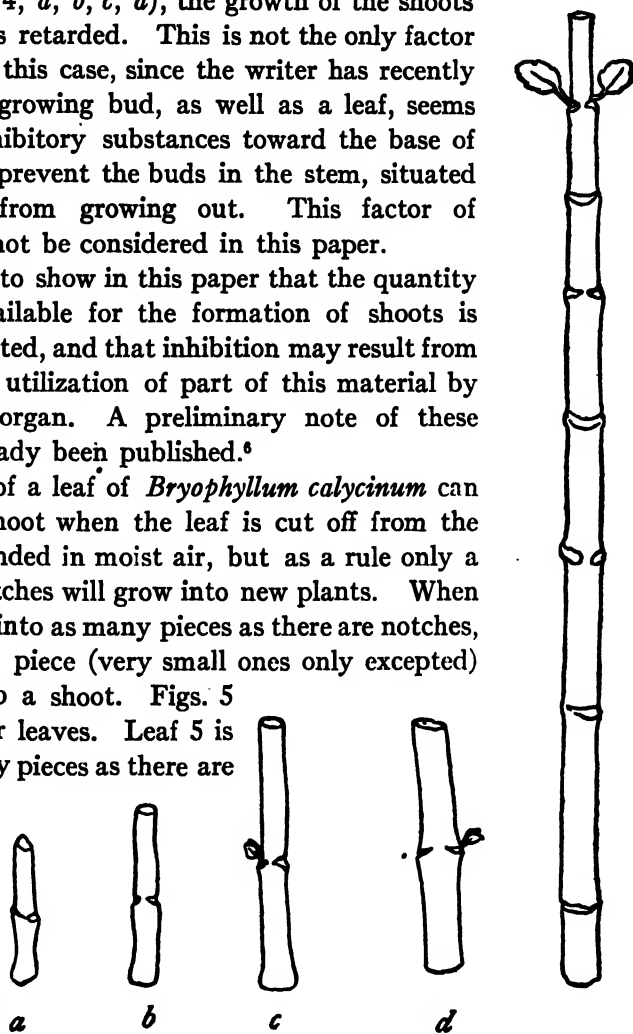


FIG. 4.—Shows that inhibited basal part of the long stem accelerates growth of the two apical buds, since in pieces with one node only (*a*, *b*, *c*, *d*) the buds do not grow at all, or much more slowly.

<sup>5</sup> LOEB, J., Science 46: 547. 1917.

<sup>6</sup> *Ibid.*, 45: 436. 1917.



in practically each notch, while leaf 6 has formed only 4 shoots. We assume that in the latter leaf the shoots which grow out first inhibit the growth in the other notches. (No part of the leaf of *Bryophyllum calycinum* except the notches is able to give rise to shoots or roots. The formation of roots will be omitted from consideration in this paper in order to simplify the discussion.) Our contention is that this inhibition in leaf 6 is due to the absorption of all the material available for shoot formation by the 4 notches that happened to grow out first, thus depriving the other notches of the material

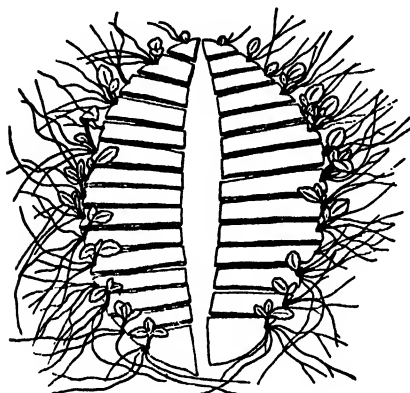


FIG. 5.

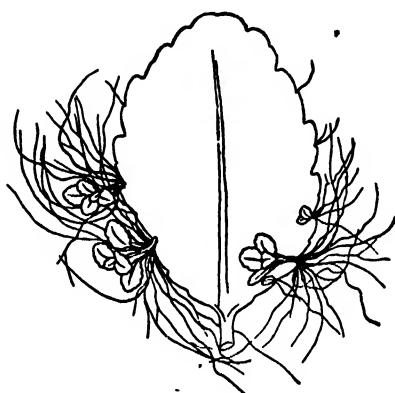


FIG. 6.

FIGS. 5, 6.—Sister leaves: fig. 5, leaf cut into as many pieces as notches; almost every notch forms a shoot; fig. 6, leaf intact, only 4 shoots formed, 3 being considerably larger than those shown in fig. 5, thus indicating tendency of both leaves to produce equal masses of shoots, although number of shoots may vary considerably.

needed for the growth of shoots. By comparing figs. 5 and 6 it will be noticed that 3 of the shoots which leaf 6 produced are considerably larger than the individual shoots of leaf 5, and this suggests the possibility that the isolation of a piece with one notch simply prevents the material needed for the growth of the notch being taken away by some of the other notches which by chance start growing a little earlier.

In order to prove this we must be able to show that if we isolate two sister leaves (which are of equal size, age, and history) and keep them under equal conditions, they will produce in equal times

approximately equal masses of shoots. It is necessary, of course, that both leaves are healthy and not yet beginning to etiolize, and that they should not do so during the course of the experiment. It is necessary also that the experiment be continued long enough (that is, a month or longer at about 23°C.) to allow the shoots to reach a sufficiently large size, since if the shoots are too small the error in measuring their masses prevents exact results. On the other hand, the experiment must not last too long, for if the shoots become too large they produce themselves too considerable a share of the material needed for their own growth. The leaves were generally kept on wet filter paper in flat dishes with a loose glass cover. One of the greatest sources of error or variation in the results was probably the differences in the absorption of water by the roots of different leaves or pieces of leaves. Furthermore, light is an important factor in determining the masses of shoots produced, and when leaves are suspended in an aquarium and able to shade each other, inequality of illumination of sister leaves also forms a source of error. The new shoots can be cut off from the leaf comparatively neatly, although slight variations or errors are unavoidable in this operation. The shoots were freed from water droplets on their surface and weighed fresh, on the assumption that the dry weight under the conditions of the experiment is a fairly constant fraction of the fresh weight, which has been found to be approxi-

TABLE I.

Sister leaves		Number of shoots produced from leaf	Mgm. of shoots pro- duced in 33 days
I.	Leaf 1.....	3	350
	Leaf 2.....	3	345
II.	Leaf 1.....	1	290
	Leaf 2.....	2	306
III.	Leaf 1.....	2	375
	Leaf 2.....	4	385
IV.	Leaf 1.....	5	594
	Leaf 2.....	4	607
V	Leaf 1.....	4	457
	Leaf 2.....	5	455

mately correct. The leaves were usually but not always weighed without their petioles.

Table I gives the weight of the shoots produced by 5 pairs of sister leaves in 33 days (February 15—March 20). The two sister leaves are always designated as 1 and 2. It is found that each of two sister leaves which were of equal size produced almost identical masses of shoots in the same period of time and under equal conditions, although the number of shoots by two sister leaves differed.

TABLE II.  
*March 29—April 20, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
I. { Leaf 1.....	7	0.2560	2.3030	111
{ Leaf 2.....	9	0.2455	2.2555	109
II. { Leaf 1.....	5	0.1920	1.783	108
{ Leaf 2.....	4	0.2075	1.8735	111
III. { Leaf 1.....	5	0.2005	2.262	89
{ Leaf 2.....	3	0.1605	1.982	81
IV. { Leaf 1.....	5	0.1910	1.668	114
{ Leaf 2.....	4	0.1570	1.402	112
V. { Leaf 1.....	4	0.3205	2.5125	128
{ Leaf 2.....	7	0.3760	3.0770	122
VI.* { Leaf 1.....	3	0.1790	2.191	82 etiolized
{ Leaf 2.....	?	0.0595	1.597	37 leaves
VII. { Leaf 1.....	6	0.2355	2.6495	89
{ Leaf 2.....	4	0.216	2.288	94
VIII. { Leaf 1.....	2	0.109	1.326	82
{ Leaf 2.....	4	0.132	1.505	88
IX. { Leaf 1.....	3	0.172	1.927	89
{ Leaf 2.....	5	0.187	2.093	89
Average { Leaves 1.....		1.675	16.430	102
{ Leaves 2.....		1.682	16.476	102

\* Pair VI is not included in the average.

Table II gives another experiment of the same kind. The two sister leaves produce in each case almost identical masses of shoots in the same time, although the number of shoots varies quite often. The shoots produced by the two leaves of the sixth pair differ considerably, but those two leaves were etiolized. They were excluded from the calculation of the average shoot production, which is exactly the same for each set of leaves, namely 102 mgm. of shoots for 1 gm. of leaf.

Tables III and IV show a slightly greater variation than tables I

TABLE III.  
*April 11—May 10, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
I. { Leaf 1.....	2	0.180	1.655	109
{ Leaf 2.....	1	0.201	1.590	126
II. { Leaf 1.....	2	0.115	1.050	109
{ Leaf 2.....	2	0.166	1.505	110
III. { Leaf 1.....	3	0.155	1.081	143
{ Leaf 2.....	2	0.140	1.098	127
IV. { Leaf 1.....	3	0.123	1.158	106
{ Leaf 2.....	3	0.126	1.245	101
V. { Leaf 1.....	2	0.110	1.038	106
{ Leaf 2.....	2	0.089	0.995	90
VI. { Leaf 1.....	2	0.183	1.646	111
{ Leaf 2.....	2	0.153	1.383	111
VII. { Leaf 1.....	3	0.231	1.617	143
{ Leaf 2.....	3	0.178	1.463	122
VIII. { Leaf 1.....	4	0.220	1.547	142
{ Leaf 2.....	2	0.146	1.172	125
IX. { Leaf 1.....	3	0.119	1.230	97
{ Leaf 2.....	3	0.149	1.410	106
Average { Leaves 1.....		1.436	12.022	119
{ Leaves 2.....		1.348	11.861	114

and II, owing to the inevitable errors in such experiments (errors in cutting off and ascertaining the weight of the small shoots, errors in evaporation, differences in the condition of the two sister leaves, and in the external conditions of moisture and light, and others). The fact that these errors are accidental is proved by the proximity of the average shoot production in each set of leaves, which is 119

TABLE IV.

*Intact Sister Leaves; March 20—April 18, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf in 29 days
I. { Leaf 1.....	3	0.127	1.310	97
{ Leaf 2.....	2	0.128	1.170	109
II. { Leaf 1.....	2	0.150	1.595	94
{ Leaf 2.....	3	0.1325	1.323	100
III. { Leaf 1.....	4	0.2085	1.9175	109
{ Leaf 2.....	2	0.1575	1.722	91
IV. { Leaf 1.....	3	0.270	2.286	118
{ Leaf 2.....	4	0.145	1.586	91
V. { Leaf 1.....	2	0.147	1.3385	110
{ Leaf 2.....	5	0.2075	2.061	101
VI. { Leaf 1.....	4	0.211	1.9735	107
{ Leaf 2.....	3	0.220	2.0275	107.5
VII. { Leaf 1.....	2	0.1065	0.9435	113
{ Leaf 2.....	3	0.105	1.062	99
VIII. { Leaf 1.....	5	0.233	2.332	100
{ Leaf 2.....	4	0.228	2.2595	101
Average { Leaves 1.....		1.452	13.69	106
{ Leaves 2.....		1.322	13.21	100

and 114 mgm. of shoots per gm. of leaf in table III, and 106 and 100 mgm. in table IV.

We may make the following statement, therefore: *Two healthy, isolated sister leaves of equal mass will produce in equal times and under equal conditions approximately equal masses of shoots, although*

*the number of shoots in the two leaves may differ.* The variations in the results lie within the limits of the unavoidable errors of the experiments.

It would follow that if we cut a leaf into two symmetrical halves

TABLE V.  
*April 12—May 15.*

	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
20 left halves of leaves.....	33	2.916	19.307	151
20 right halves of leaves.....	31	2.790	18.466	151

TABLE VI.  
*Sister Leaves, Each Cut into Two Symmetrical Halves; April 3—May 4.*

Sister leaves			Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
I.	Leaf 1	Left half.....	2	0.188	0.936	203
		Right half.....	2	0.183	0.959	191
	Leaf 2	Left half.....	1	0.202	1.009	200
		Right half.....	2	0.254	1.241	205
II.	Leaf 1	Left half.....	1	0.057	0.427	133
		Right half.....	2	0.053	0.398	133
	Leaf 2	Left half.....	1	0.063	0.441	143
		Right half.....	1	0.056	0.398	141
III.	Leaf 1	Left half.....	1	0.120	0.820	146
		Right half.....	3	0.111	0.758	146
	Leaf 2	Left half.....	1	0.116	0.713	163
		Right half.....	1	0.115	0.721	160
IV.	Leaf 1	Left half.....	1	0.070	0.497	141
		Right half.....	1	0.072	0.580	124
	Leaf 2	Left half.....	2	0.073	0.595	122
		Right half.....	1	0.068	0.522	130

each half should produce equal masses of shoots in the same time and under the same conditions. This is approximately correct, as table VI shows.

TABLE VII.

*Sister Leaves, One Intact, the Other Cut into Four Pieces; April 18—May 18, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
I. { Leaf 1, intact.....	2	0.198	1.170	169
{ Leaf 2, 4 pieces.....	4	0.2025	1.205	168
II. { Leaf 1, intact.....	2	0.216	1.596	135
{ Leaf 2, 4 pieces.....	4	0.214	1.560	137
III. { Leaf 1, intact.....	1	0.305	1.925	158
{ Leaf 2, 4 pieces.....	4	0.368	2.110	174
IV. { Leaf 1, intact.....	2	0.340	1.9015	177
{ Leaf 2, 4 pieces.....	4	0.2635	1.475	179
V. { Leaf 1, intact.....	2	0.197	1.072	184
{ Leaf 2, 4 pieces.....	4	0.200	1.227	163
VI. { Leaf 1, intact.....	3	0.265	1.743	152
{ Leaf 2, 4 pieces.....	6	0.292	1.675	174
VII. { Leaf 1, intact.....	2	0.2415	1.741	138
{ Leaf 2, 4 pieces.....	4	0.255	1.745	146
VIII. { Leaf 1, intact.....	1	0.195	1.260	155
{ Leaf 2, 4 pieces.....	4	0.109	0.660	165
IX. { Leaf 1, intact.....	2	0.218	1.198	182
{ Leaf 2, 4 pieces.....	4	0.209	1.110	188
X. { Leaf 1, intact.....	2	0.223	1.514	147
{ Leaf 2, 4 pieces.....	4	0.180	1.280	140
XI. { Leaf 1, intact.....	4	0.258	1.820	142
{ Leaf 2, 4 pieces.....	5	0.2615	1.818	144
XII. { Leaf 1, intact.....	2	0.227	1.498	151
{ Leaf 2, 4 pieces.....	3	0.191	1.205	158
Average { Intact leaves.....	25	2.884	18.435	156
{ Leaves cut into 4 pieces.....	50	2.747	17.070	161

The experiment was repeated (table V), and we may confine ourselves to a statement of the average result. The two halves are designated as right and left, when facing the observer with their basal end and when lying on their lower side.

It is obvious, therefore, that if leaves are cut symmetrically, the two halves will produce in equal times and under equal conditions on the average exactly the same mass of shoots, even when the number of shoots in the two halves varies.

While in the preceding experiments the number of shoots produced in sister leaves was not identical, yet it seemed of interest to find out whether the law of the production of equal masses of shoots by equal masses of sister leaves was true also if the number of shoots produced in the two leaves differed considerably. For this purpose one leaf was cut into 4 pieces while its sister leaf remained intact. The whole leaves produced fewer shoots than the leaves cut into 4 pieces; nevertheless, the masses of shoots produced in the two sets of leaves remained the same. Thus 12 intact leaves produced 25 shoots, while their sister leaves cut into 4 pieces each produced 50 shoots. Yet the average weight of shoots produced per gm. of leaf was 156 mgm. for the intact leaves and 161 mgm. for the leaves cut into 4 pieces, in spite of the difference in the number of shoots produced. Table VII gives the results in detail. These experiments again confirm the conclusion that equal masses of sister leaves produce equal masses of shoots in equal time, even if the number of shoots in the two cases is in the ratio of 1:2.

In order to test further this law it seemed necessary to modify the experiment. For this purpose the mass of one of two sister leaves was reduced by cutting out a large piece from the center, leaving the edge intact (fig. 8), while the other leaf remained intact (fig. 7). If the law just expressed is correct, it should follow that the mass of shoots produced by such sister leaves (one set of which remained intact while the mass of the other set was reduced by cutting out pieces from the middle) would no longer be equal, but would differ in proportion to the mass of the two sets of leaves. This was found to be approximately true, as table VIII indicates.

Thus in experiment I (table VIII) the 5 intact leaves weighing 13.8 gm. produced in 37 days 1405 mgm. of shoots, while their 5



sister leaves, whose weight was reduced from approximately 13.8 gm. to 7.6 gm. (by cutting out pieces from the center of the leaf as indicated in fig. 8), produced in the same time and under the same condition 755 mgm. of shoots. While the proportion of the mass of the two sets of leaves was  $\frac{7.6}{13.8}$ , the proportion of the mass of the shoots produced was  $\frac{755}{1408}$ . These two quotients are almost identical.

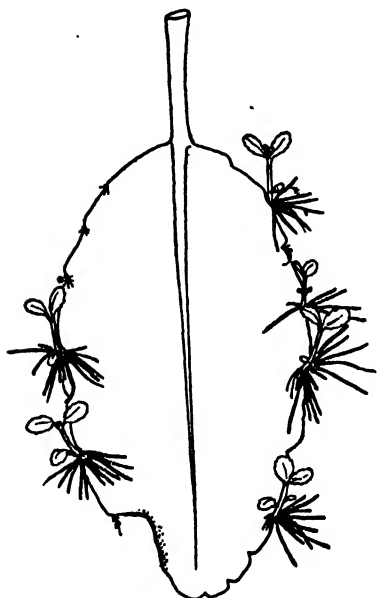


FIG. 7.

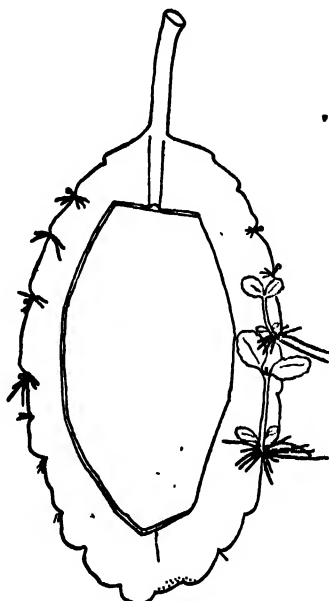


FIG. 8.

FIGS. 7, 8.—Sister leaves suspended in moist air: fig. 7, leaf intact; fig. 8, leaf with mass reduced by cutting out large piece from center of leaf; mass of shoots produced smaller than that produced by intact leaf; drawn 23 days after beginning of experiment.

The same is true for experiments II, III, and V, while in IV there is a greater discrepancy. Experiments III and IV indicate that if there is such a discrepancy it seems to be in favor of the leaf reduced in size. Since light plays such an important rôle in the production of shoots the discrepancy may possibly be due to the accidental fact that the intact leaves shaded each other more in these experiments than the leaves with their centers cut out.

TABLE VIII.

Number of experiment	Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots produced per gm. of leaf
I.	Leaves dipping (a) 5 leaves, with in water, duration of experiment 37 days center cut out.....	11	0.755	7.61	99
	(b) 5 sister leaves, intact.....	9	1.405	13.80	101
II.	Leaves dipping (a) 7 leaves, with in water, duration of experiment 25 days center cut out.....	21	1.213	9.899	122
	(b) 7 sister leaves, intact.....	25	1.995	16.935	118
III.	Leaves dipping (a) 9 leaves, with in water, duration of experiment 32 days center cut out.....	22	2.292	10.522	218
	(b) 9 sister leaves, intact.....	30	3.430	17.852	192
IV.	Leaves dipping (a) 12 leaves, with in water, duration of experiment 27 days center cut out.....	33	2.175	11.245	194
	(b) 12 sister leaves, intact.....	33	2.761	19.395	142
V.	Leaves kept in (a) 5 leaves, with moist air, duration of experiment 38 days center cut out.....	13	0.690	5.42	109
	(b) 5 sister leaves, intact.....	20	1.207	11.81	102

*The mass of shoots produced by the whole leaves and by the leaves reduced in mass, therefore, were approximately in proportion with the masses of the two sets of leaves; or in other words, each set of sister leaves produced approximately the same weight of shoots per gram of leaf in the same length of time.*

When the leaf is isolated and put on moist filter paper or if it is suspended in moist air, as a rule more than one notch grows out into a shoot (fig. 6). This seems to indicate that the material available for shoot formation in one leaf does not all flow easily into one notch, so that we should expect that the material available in a leaf might be utilized more completely if the leaf were cut into several smaller pieces than if all the material had to go into one shoot only. This fact is evident from the following experiment.

In one leaf the whole edge (containing the notches) with the exception of one notch was removed (fig. 9). Such a leaf could form only one shoot. The sister leaf was cut into 4 pieces but the edges



FIG. 9.—Sister leaves: one cut into 4 pieces, other not subdivided, but all notches except one removed; from this notch a shoot is produced considerably larger than each of shoots produced from the 4 smaller pieces of other leaf; photographed 19 days after beginning of experiment.

were left intact. These 4 pieces could form at least 4 shoots. Fig. 9 shows such a pair of sister leaves. It was to be expected that

the total weight of the shoots formed by the 4 pieces would be approximately equal to that of the one shoot in the sister leaf, or exceed it slightly for the reason indicated. Table IX shows that 6 shoots produced in 6 whole leaves differed very little in weight from the 32 shoots produced by their 6 sister leaves, each of which was

TABLE IX.

*Sister Leaves: (a) Whole Leaf, but All Notches with Exception of One Removed; (b) Cut into 4 Pieces, but No Notch Removed; April 5-April 25, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots per 1 gm. of leaf
I. { (a) Whole leaf. ....	1	0.1935	2.403	81
(b) 4 pieces. ....	6	0.206	2.267	91
II. { (a) Whole leaf. ....	1	0.110	2.234	49
(b) 4 pieces. ....	6	0.105	2.431	43
III. { (a) Whole leaf. ....	1	0.136	1.647	83
(b) 4 pieces. ....	5	0.185	2.083	89
IV. { (a) Whole leaf. ....	1	0.196	1.8325	107
(b) 4 pieces. ....	7	0.2975	2.387	125
V. { (a) Whole leaf. ....	1	0.201	2.035	99
(b) 4 pieces. ....	4	0.246	2.225	110
VI. { (a) Whole leaf. ....	1	0.110	1.086	101
(b) 4 pieces. ....	4	0.154	1.4015	109
	Total number of shoots	Total weight of shoots	Total weight of leaves	Shoots per gm. of leaf; mgm.
Average { (a) Whole leaves. ....	6	0.9465	11.237	84
(b) Cut leaves. ....	32	1.193	12.794	93

cut into 4 pieces, but that the difference was in favor of the leaves cut into 4 pieces. The latter produced per gram leaf 93 mgm. of shoots, the former 84 mgm. In a second set of experiments the difference was in the same direction, but a little larger, namely 98 mgm. and 74.5 mgm. (table X). While these experiments confirm the law of equal production of shoots by equal masses of leaf, they also indicate that several shoots can consume the material available in one leaf more quickly than if only one shoot is present.

A second complication is encountered when small pieces containing one notch are cut out from a leaf (fig. 5). In this case it may happen that when the piece is too small the notch of the small piece may not form any shoot at all, or the growth may be materially delayed. This is intelligible on the assumption that if the quantity of material available falls below a certain minimum no shoot can grow out. Fig. 10 illustrates this statement. A large and a small

TABLE X.

*Sister Leaves: (a) Whole Leaf, but All Notches with Exception of One Removed; (b) Cut into 4 Pieces, but No Notch Removed; April 4-April 25, 1917.\**

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots per 1 gm. of leaf
I. { (a) Whole leaf. ....	1	0.201	2.202	90.5
(b) 4 pieces. ....	6	0.316	2.542	124
II. { (a) Whole leaf. ....	1	0.144	2.0325	71
(b) 4 pieces. ....	4	0.2335	2.3235	100.5
III. { (a) Whole leaf. ....	1	0.162	1.832	88
(b) 4 pieces. ....	4	0.179	1.950	92
IV. { (a) Whole leaf. ....	1	0.147	2.152	68
(b) 4 pieces. ....	4	0.256	2.5145	102
V. { (a) Whole leaf. ....	1	0.150	2.710	55
(b) 4 pieces. ....	4	0.191	2.667	72
VI. { (a) Whole leaf. ....	1	0.084	0.986	85
(b) 4 pieces. ....	4	0.111	1.107	100
	Total number of shoots	Total weight of shoots	Total weight of leaves	Shoots per gm. of leaf; mgm.
Average { (a) Whole leaves. ....	6	0.8889	11.915	74.5
(b) Cut leaves. ....	26	1.2875	13.104	98

piece were cut out from the same leaf, each piece containing one notch only, the notches in each set of two pieces originally being symmetrical. The photograph was taken 36 days after the beginning of the experiment. It will be seen that the size of the shoot varies with the size of the piece, but that some of the smallest pieces

have failed to form shoots. This fact is to be considered in experiments in which one leaf is left intact and the sister leaf cut into



FIG. 10.—Large and very small pieces, each with one notch cut from one leaf; smallest pieces have not yet formed shoots (in 4 weeks); parallelism between size of leaf and size of shoot obvious.

as many pieces as there are notches. In that case it may happen that the law of equal production of shoots by equal masses of leaves may not hold strictly, for two reasons: (1) some of the small pieces may not form any shoot at all or form it only too late; (2) a compli-

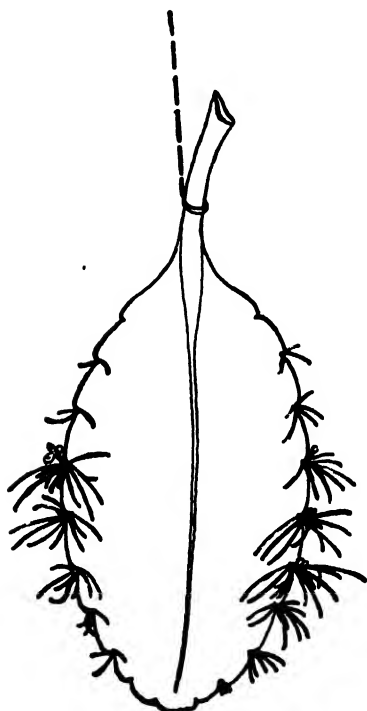


FIG. 11.

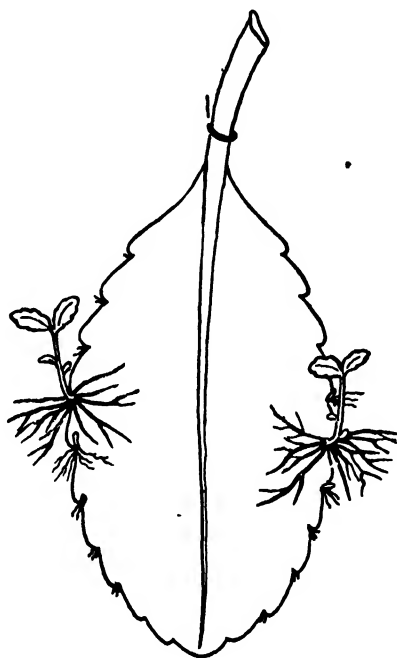


FIG. 12.

FIGS. 11, 12.—Same leaf suspended in moist air, in fig. 11 after 18 days, in fig. 12 after 28 days; at first all notches in middle of leaf form roots and in some of them shoots begin to develop (fig. 11); later (fig. 12) only two of these shoots in middle of leaf grow, while roots in other notches not only ceased to grow but are shriveled up; proves inhibiting effect of most rapidly growing notches on others.

cation may vitiate the result in the opposite direction, namely, that the shoots formed by small pieces can use the material available for shoot formation more readily than the shoots in the whole leaves. Table XI gives the results of such an experiment on 3 pairs of sister leaves, one leaf remaining intact or cut into two symmetrical halves, while the other was cut into as many pieces as there were notches.

In spite of the enormous difference in the number of shoots in both cases, the weight of shoots produced by one gram leaf in a given time was not very different, the average being 143 mgm. of shoots in one set and 150 mgm. in the other set per gram of leaf.

The law of equal production of shoots by equal masses of leaves explains why the shoots growing out from the notches of a leaf grow the more rapidly the smaller their number. It does not explain how it happens that in an isolated leaf not all the notches grow out into shoots.

TABLE XI.  
*February 15—March 20, 1917.*

Sister leaves	Number of shoots	Weight of shoots in gm.	Weight of leaves in gm.	Mgm. of shoots per gm. of leaf
I. { (a) 2 halves.....	3	0.316	1.866	170
(b) 16 pieces.....	14	0.345	1.727	200
II. { (a) Whole leaf.....	4	0.490	2.061	233
(b) 14 pieces.....	14	0.312	1.810	172
III. { (a) Whole leaf.....	2	0.450	4.465	100
(b) 17 pieces.....	15	0.300	3.17	95
Averages { Whole or half leaves.....	9	1.256	8.392	150
Leaves cut into small pieces.....	43	0.957	6.71	143

When we cut off a leaf and suspend it in moist air (the air not being completely saturated with water vapor), after some time most of the notches form roots, as the leaf in fig. 11 indicates, which was drawn 18 days after the beginning of the experiment. If there are any notches which do not form roots, they are usually found at the apex and at the base of the leaf (fig. 11). After the roots are formed, shoots begin to grow out of the notches, and now a remarkable change occurs. Fig. 12 shows the same leaf as fig. 11, 10 days later. Two of the shoots in the notches in the middle of the leaf have grown into shoots, and in these notches the roots have continued to grow; while the roots formed in the other notches have shriveled up and no new shoots have grown out.



From this observation, which is typical and which has been verified many times, we are inclined to draw the following conclusion. As long as the leaf is part of the normal plant, its sap flows into the stem of the plant and the notches cannot grow out. When the leaf is separated from the plant and suspended in moist air, this flow ceases and the material carried in the form of sap remains in the leaf and becomes available for the notches. As a consequence

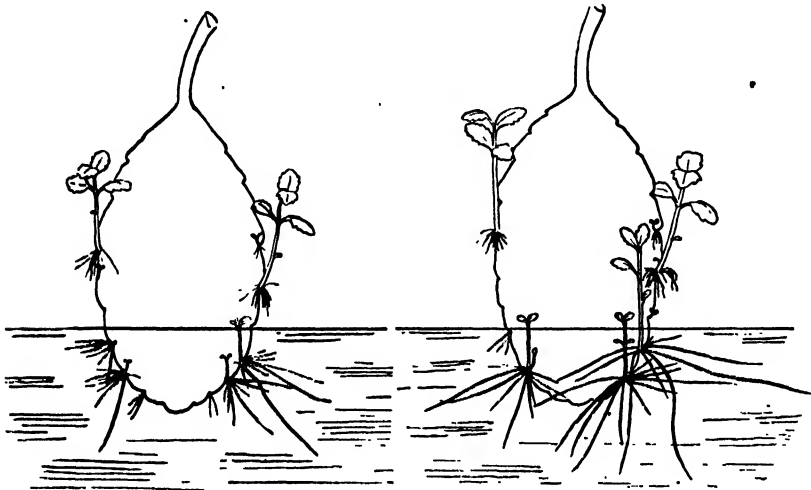


FIG. 13.

FIG. 14.

FIGS. 13, 14.—Same leaf as in figs. 11 and 12, after 45 and 52 days; on 33d day leaf was dipped with apex in water and now new shoots are formed in watered notches, which grow rapidly and soon reach size of two original shoots; proves that amount of water determines which notches shall grow into shoots.

the notches in the leaf begin to grow out. The chances for growth are apparently not equal for all the notches of a leaf suspended in moist air, but are as a rule better for those in the middle of the leaf, where the leaf is thicker and where probably more sap is available. The roots grow out before the shoots begin to grow. Those shoots which happen to grow out first now become a center of attraction for all the material available for growth in the leaf, and they thereby inhibit not only the growth in most of the other notches but actually cause the roots formed in other notches to dry out again, as a comparison of fig. 12 with fig. 11 shows. We cannot

yet tell how it happens that the more rapidly growing leaf attracts the sap to itself.

We have mentioned that as a rule the notches which will grow out first are not the ones at the apical or basal ends, but in the middle of the leaf, where the leaf is thickest and where apparently more sap is available. That it is possibly only the quantity of water which decides the initiation of growth<sup>7</sup> is suggested by the fact that a leaf, like the one in fig. 12, which, when suspended in moist air forms no shoots in the apical notches, can be caused at any time to form new shoots in these notches if we let the apex dip into water. As soon as this happens these notches will form shoots and these shoots will soon equal or exceed in size the old stems, and in turn may now inhibit the growth of the latter.

The leaf in fig. 12 was drawn on January 30. On February 7 its apex was suspended in water and soon new shoots formed in the apical notches (figs. 13, 14). Fig. 13 was drawn 9 days, and fig. 14, 16 days after the apex was put into water. It will be noticed that new shoots have grown out from three of the apical notches dipping in water. This never happened when the leaves remained in moist air. It can be shown that such a leaf when dipping in water absorbs water, and we are justified therefore in assuming that the in-

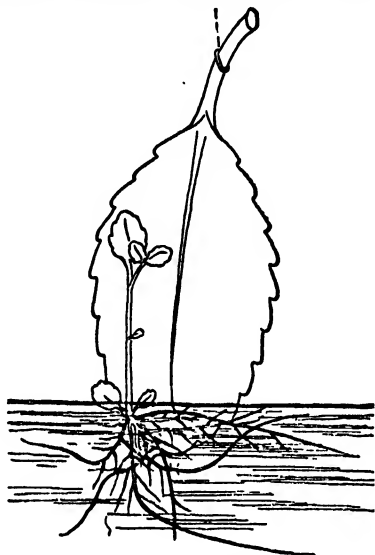


FIG. 15.—Leaf dipped with apex in water; drawn after 28 days: in such cases the shoot from one of watered notches will grow out so rapidly that as a rule it suppresses root and shoot formation in notches in middle of leaf, where growth is most rapid, when leaf is suspended in moist air, as comparison of figs. 15 and 11 will show.

<sup>7</sup> This refers only to the initial step of starting the growth in a dormant bud; its actual growth, of course, depends upon the supply of sugar, amino acids, salts, and other solutes from the leaf.

crease in the contents of water in a notch or the starting of a current of water through the notch starts its growth.

We may compare the conditions for the initiation of the growth of a notch in a leaf to those of the growth of a seed, inasmuch as in both cases an absorption of water is necessary to initiate growth.

In both cases the water may play the rôle of accelerating the velocity of certain chemical processes which are needed for the formation of roots and shoots.

The experiment just described never fails, and we may therefore say with some justification that *in an isolated leaf suspended in moist air those notches will grow out first which by chance have at first the necessary supply of water (or of sap in general)*. Those shoots which grow out first will then automatically inhibit the growth of the other notches by drawing the solutes and the water toward themselves.

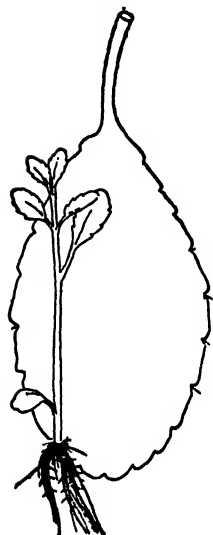


FIG. 16.—Same leaf as fig. 15: on 33d day leaf was removed from water and suspended in moist air; rapidly growing old shoot prevents any further growth in other notches.

This view is supported by another set of experiments. In the previous experiment the isolated leaves were first suspended in moist air and afterward allowed to dip into water. When we let the apex of the isolated leaf dip from the beginning into water, only those notches will give rise to shoots which are just under the level of the water or just above it (fig. 15). Such shoots grow more rapidly than the shoots of leaves suspended entirely in moist air, and this fact also suggests that it is the quantity of water which decides which notches grow out first. It is also noticeable that when an isolated leaf dips into water from the beginning the notches in the middle of the leaf, which would have given rise to roots (fig. 11) if the leaf had been suspended entirely in air, now generally fail to do so (fig. 15), if the leaf is not too large, presumably because the greater rate of growth of the notch dipping into water inhibits the growth of roots in the rest of the notches. With the greater rate of growth of a notch is

linked a greater inhibiting power upon the growth of the other notches, inasmuch as the flow of sap is directed toward a rapidly growing notch. The leaf in fig. 15 was then taken out of water and suspended in air on February 4. No new notches grew out, as was so be expected. The rapidly growing original shoot attracted all the sap available. A few roots started in some of the notches, but shriveled up almost as soon as they were formed (fig. 16). The results of this experiment are as constant as those of the previously mentioned experiment.

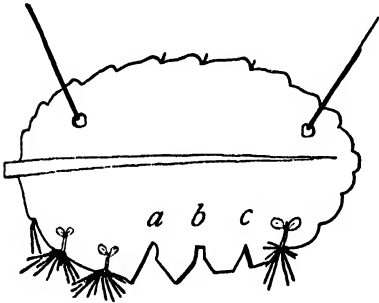


FIG. 17.

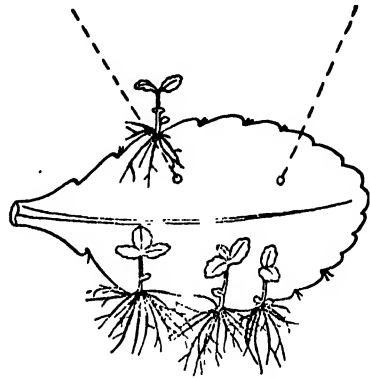


FIG. 18.

FIGS. 17, 18.—Leaves suspended in moist air with main axis in horizontal position: shows formation of shoots is favored on lower side, where water is bound to collect in larger masses; in fig. 17 notches at *a*, *b*, *c* had been removed.

These observations thus give us a rather clear view of the mechanism of correlation in an isolated leaf. In order to start the growth of a notch it is necessary that a stream of water should reach the notch. This will not happen as long as the leaf is part of a stem. Only when the leaf is old, ready to drop from the plant, do we notice occasionally that a shoot may form in the notches of a leaf while it is still attached to the plant, but this is rare. We can start the growth of notches at will, however, when the leaf is cut off. In that case that notch or those notches will grow first which happen to receive the greatest water supply (from within or without). Those which begin to grow more rapidly than the rest will automatically cause a current of sap toward themselves, in a way

not yet understood. They thereby inhibit or retard the growth in the other notches. This inhibition can be overcome at any time by supplying more water to an inhibited notch from without, whereby we accelerate the rate of chemical reactions in this notch, which in turn will now cause a flow of sap toward itself, but we can also increase the flow of sap to certain notches from within. The writer's former observations have shown that the sap in the leaf can flow around a corner, a fact which suggests the existence of many interlocking channels for the sap flow. It occurred to us that if we suspend such leaves in moist air with their longitudinal axes put hori-

TABLE XII.

	SHOOTS PRODUCED		WEIGHT OF LEAVES IN GM.	MG. OF SHOOTS PER GM. OF LEAF
	Number	Weight in gm.		
I. 6 pairs of leaves suspended in moist air				in 30 days
In dark.....	3	0.016	11.65	1
In light.....	24	0.543	8.03	68
II. 7 pairs of leaves dipping in water				in 26 days
In dark.....	14	0.406	13.377	30
In light.....	17	1.725	17.270	100

zontally (figs. 17, 18), the notches on the lower side of the leaf should form more shoots than the notches on the upper side, since the sap should collect in larger masses on the lower edge of the leaf. This is apparently the case, since very often shoots form only on the lower side of such a leaf, as in fig. 17 (where the notches in *a*, *b*, *c* had been removed before the experiment began). In fig. 18 three notches formed on the lower and one on the upper side. The experiment just mentioned and which has often been repeated supports the idea that the first shoots grew out where the water or sap collects, the water naturally having the tendency to flow downward. Light is an important factor in the shoot production of the leaf of *Bryophyllum calycinum*. Isolated leaves kept in the dark produce a considerably smaller mass of shoots than their sister leaves kept in light, as the following experiment shows. Six leaves taken from different plants or nodes were suspended in the dark, either

in moist air or were dipped with their apices in water; while their sister leaves were suspended in the same way but in light. Table XII shows the difference in the amount of shoot production.

It is obvious that in both cases the shoot formation is considerably greater in the light than in the dark. The experiment seems to indicate that either the process of assimilation contributes directly or indirectly to the formation of material for shoots in the leaf, or that the light in some other way contributes to the shoot formation. It is obvious that among the conditions which are to be considered in the production of equal masses of shoots by equal masses of leaves equality of illumination is of special importance. The writer observed deviations from the rule of equal production of shoots by equal masses of sister leaves when the leaves were able to partially cover or shade each other.

In this paper we have considered only the production of equal masses of shoots by equal masses of sister leaves of *Bryophyllum calycinum*. The law is probably correct for leaves of *Bryophyllum* in general, provided a sufficiently large number of leaves are compared, so that the influence of individual differences in the leaves (age, amount of chlorophyll, etc.) is eliminated.

It is also very probable that this form of correlative inhibition of growth is not confined to the leaf of *Bryophyllum*, but is a more general phenomenon. Thus it seems to exist in the potato, where the growth of one bud seems to inhibit the growth of other buds of the same tuber, and perhaps for reasons similar to those set forth here.

#### SUMMARY.

1. Equal masses of sister leaves produce approximately equal masses of shoots in equal time and under equal conditions, even if the number of shoots varies considerably.

2. Those shoots which grow out first attract automatically the material available for shoot formation, thus withholding it from the other buds; the mechanism of this automatic attraction is not yet known.

3. These two factors, the limited amount of material available for growth and the automatic attraction of the material by the buds

which grow out first, explain the inhibiting effect of these buds on the growth of the other buds.

4. The relative amount of water in a notch determines which notches give rise to shoots first; by furnishing a liberal water supply from without or from within we can determine at will which notches shall grow out first.

## IONIZATION OF PROTEINS AND ANTAGONISTIC SALT ACTION.

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### I.

In 1899 Wolfgang Pauli<sup>1</sup> and the writer<sup>2</sup> independently reached the conclusion that electrolytes when acting on proteins formed ion-protein compounds. The writer anticipated that these ion-proteins would explain the mystery of many life phenomena. He was especially interested in one of the most universal physiological actions of salts; namely, the antagonistic salt action, for which the annihilation of the effects of a high concentration of a salt with univalent cation, *e.g.* NaCl, by a low concentration of a salt with bivalent cation, *e.g.* CaCl<sub>2</sub>, is perhaps the best known example. Although he and many others tried to demonstrate this type of antagonism in proteins they never succeeded. It was a further disappointing fact that Hardy<sup>3</sup> found that globulins apparently form electrically neutral compounds with neutral salts and this seemed to harmonize with the older observations of Liebermann and Bugarszky. Pauli<sup>4</sup> had expressed the idea that a low concentration of salts ionizes globulins and thereby causes their solution, but even he assumed not a real chemical combination but adsorption between the globulin and the salt.

Meanwhile, many workers, and especially Pauli and his pupils, had developed a number of methods for discriminating between the chemical behavior of ionized and non-ionized proteins, but the surest method of producing ionized proteins was to treat proteins with either acid or base. It was generally found that when a protein with little tendency to electrolytic dissociation is treated with acid

<sup>1</sup> Pauli, W., *Arch. ges. Physiol.*, 1899, lxxviii, 314.

<sup>2</sup> Loeb, J., *Arch. ges. Physiol.*, 1899, lxxv, 303; *Am. J. Physiol.*, 1900, iii, 327.

<sup>3</sup> Hardy, W. B., *J. Physiol.*, 1905-06, xxxiii, 251.

<sup>4</sup> Pauli, *Fortschr. naturwiss. Forschung.*, 1912, iv, 223.



of the right concentration a protein salt is formed which undergoes a strong electrolytic dissociation, while the addition of a neutral salt diminishes this degree of dissociation, as was to be expected. Loeb and Wasteneys,<sup>5</sup> and later the writer alone,<sup>6</sup> were able to show that the toxic effect of acid can be diminished or suppressed by the addition of neutral salts. This was the first direct support of the idea that antagonistic salt action was due to a transformation of ionized protein into electrically neutral protein.

The second case where it seemed possible to attribute antagonistic salt action to a transformation of ionized into non-ionized protein consisted in the observation that the diffusion of a low concentration of KCl into the egg of *Fundulus* is impossible or extremely slow when this salt is alone in solution, but is rendered possible through the addition of a small amount of a second salt, *e.g.* NaCl ("salt effect"), and that diffusion of KCl is rendered impossible again when more of the second salt is added. The analogy with the solubility of globulin suggested that the addition of a small quantity of the second salt caused an ionization of some protein in the membrane of the egg which was suppressed again by the addition of more salt; and the writer expressed this idea in a preliminary notice.<sup>7</sup> But this suggestion faces the uncomfortable fact already referred to that according to Hardy globulins form non-ionized molecules with neutral salts.<sup>8</sup>

The writer, however, has found a method which suggests strongly that the antagonism between NaCl and CaCl<sub>2</sub> is due—at least in the case to be discussed—to the fact that an ionization of protein is caused by NaCl and that this ionization or its effect upon the swelling is suppressed by a comparatively small quantity of CaCl<sub>2</sub>.

Some of the experiments have already been published.<sup>8</sup> Into a cylindrical funnel 2 gm. of finely powdered *non-bleached*<sup>9</sup> Cooper's gelatin are put; the powder is held in the cylinder by a circular piece

<sup>5</sup> Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1911, xxxiii, 489; 1912, xxxix, 167.

<sup>6</sup> Loeb, J. *Biol. Chem.*, 1915, xxiii, 139; 1917, xxxii, 147.

<sup>7</sup> Loeb, *Proc. Nat. Acad. Sc.*, 1916, ii, 511.

<sup>8</sup> Loeb, *J. Biol. Chem.*, 1917, xxxi, 343.

<sup>9</sup> Bleached gelatin as well as bleached pig's bladder does not give the same results, probably on account of an alteration in the constitution of the protein.

of filter paper. Three such funnels each with 2 gm. of gelatin are prepared. The one (I) is perfused six times in succession with 25 cc. of distilled water; the second (II) is perfused twice with 25 cc.  $m/8$  NaCl and then four times with 25 cc. of distilled water; the third (III) is perfused six times with 25 cc.  $m/8$  NaCl. In I and III a moderate swelling occurs, which soon reaches its maximum. The gelatin in II, first treated with NaCl and subsequently with distilled water, swells several hundred per cent more than either the gelatin treated only with distilled water (I) or the gelatin (III) treated only with NaCl.

The explanation of this experiment is as follows. Gelatin II forms under the influence of a comparatively high concentration of NaCl ( $m/8$  or  $m/4$ ) a compound with NaCl which is capable of ionization. This ionization is lowered through the presence of the highly concentrated NaCl solution. When, however, this latter solution is washed away by successive perfusions of the gelatin with distilled water the gelatin-NaCl compound can dissociate into gelatin and an inorganic ion, the nature of which we shall discuss later. It has been shown by Pauli and by Procter<sup>10</sup> that the swelling of protein under the influence of acid or base is due to the ionization of protein by the acid and the writer assumes that the increase in the amount in swelling of the mass of gelatin first treated with NaCl and then washed with distilled water is due to the fact that part of the gelatin is transformed into protein ions by the salt, and that this ionization and the swelling can only appear when the NaCl solution held in the capillary spaces of the powder is washed away. This statement is supported by the following facts.

1. A large number of funnels are prepared, each with 2 gm. of powdered gelatin and each is perfused twice with 25 cc.  $m/8$  NaCl. Subsequently each funnel is perfused three times with 25 cc. of a definite NaCl solution lower than  $m/8$  NaCl; namely,  $m/16$ ,  $m/32$ ,  $m/64$ ,  $m/128$ , etc., down to  $m/2048$  or lower. It is found that after three perfusions the additional swelling of the gelatin becomes noticeable in the funnel which has been washed with  $m/64$  or less concentrated NaCl solution and that the additional swelling increases rapidly with

<sup>10</sup> Procter, H. R., and Wilson, J. A., *J. Chem. Soc.*, 1916, cix, 307.

the diminution of the concentration of the NaCl solution. The explanation is that concentrations of NaCl above  $m/64$  suppress the electrolytic dissociation of the gelatin-NaCl compound to such an extent that the swelling will not exceed that caused when the gelatin is perfused permanently with  $m/8$  NaCl. Concentrations of  $m/64$  and below no longer suppress the ionization of the gelatin salt completely and the suppression is the smaller the greater the dilution of the NaCl used for perfusion. Hence the swelling of the gelatin increases rapidly with the dilution of the NaCl solution below  $m/64$  and reaches a maximum when distilled water is used for the perfusion. Table II gives adequate examples. This fact had already been published.<sup>8</sup>

2. The second proof consists of the fact that when the gelatin powder is first perfused with  $m/8$  NaCl and then with a solution of a non-electrolyte, the increasing dilution of the non-electrolyte is without effect. Table I illustrates this statement. The table gives the result of four sets of experiments. In each set about fourteen cylindrical funnels contained 2 gm. of powdered gelatin and each funnel was perfused twice with 25 cc.  $m/8$  NaCl. After this the various funnels of one series were perfused with solutions of either NaCl or glycerol or cane sugar or ethyl alcohol of increasing dilutions. The swelling was measured after three perfusions. In order to allow all the liquid not held by the gelatin (or by adhesion) to filter out the final measurement was taken after 24 hours. Precautions were taken to avoid error by evaporation. Since the cross-section of all the cylindrical funnels was the same the increase in the height of the cylindrical mass of gelatin above the height of the mass reached by the perfusion with  $m/8$  NaCl may serve as a measure of the additional swelling.

The difference between the influence of the electrolyte and the non-electrolyte is very striking. Gelatin treated with  $m/8$  NaCl does not show any further swelling when treated with  $m/8$  or  $m/16$  NaCl; from  $m/64$  on the swelling begins, gradually increasing and reaching its maximum at  $m/1024$ . Gelatin treated with  $m/8$  NaCl and then treated with solutions of non-electrolytes from 2  $m$  down swells to the same extent as if it were treated with distilled water. This harmonizes with the assumption that the swelling is determined by the degree of electrolytic dissociation of the gelatin.

3. It has been shown by Pauli and his fellow workers that ionized protein can no longer be precipitated by alcohol.<sup>4</sup> If the additional swelling of gelatin in distilled water caused by the previous treatment with  $m/8$  NaCl was due to an increase in ionization such gelatin should resist precipitation by alcohol. This is most strikingly the case. When we dissolve the three kinds of powdered gelatin, I treated with water alone, II treated first with  $m/8$  NaCl and then with water, and III treated with salt ( $m/8$  NaCl) alone, and measure the quantity of alcohol needed to precipitate 5 cc. of a 3 per cent gelatin solution of each of the three samples, we shall find that it requires about 5 cc. of 95 per cent alcohol to cause a heavy precipitate in I, and about 7 cc. to cause the same precipitate in III; while in II no precipitate is formed even by the addition of 20 cc. or more

TABLE I.

	Increase in swelling of powdered gelatin (which had first been perfused with 50 cc. m/8 NaCl) after three perfusions with 25 cc. of													
	m/4	m/2	m	m/2	m/4	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	H <sub>2</sub> O
Na Cl.....						0	0	2.5	6	15	21	33.5	42	
Glycerol.....	34	37	40	40	40	43	42	42	40	41	41	41	43	42
Cane sugar.....		35	41	49	49	51	51	50	50	51	51	51		48
Ethyl alcohol.....	42.5	48.5	54	48.5	48.5	48.5	49.5	49.5	49.5	47	47.5	47		50.5

of alcohol; the solution assumes only a bluish tint upon the addition of about 20 cc. of alcohol.

4. It was shown in the previous paper that any neutral salt with univalent cation acts like NaCl upon powdered gelatin, inasmuch as a perfusion of gelatin with a neutral salt of Li, K, or NH<sub>4</sub>, when followed by a perfusion with distilled water, causes an additional excessive swelling; while the perfusion of the powdered gelatin with neutral salts of the bivalent cations, Mg, Ca, Sr, and Ba, does not cause any additional swelling when the gelatin is afterward perfused several times with distilled water. It would, therefore, follow that gelatin treated by neutral salts with bivalent cations increases its mass of gelatin ions either not at all or only to a negligible extent. This is apparently the case. When 2 gm. of powdered gelatin are first perfused twice with  $m/8$  CaCl<sub>2</sub> and are then perfused three

times with 25 cc. of distilled water, and if then a 3 per cent gelatin solution is prepared, 5 cc. of such a solution are precipitated by slightly less than 5 cc. of alcohol (at room temperature), which is approximately the same figure as that for gelatin not treated with salt. The gelatin is, therefore, apparently not ionized by Ca.

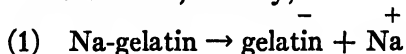
5. This difference in the action of neutral salts with bivalent and univalent cations, namely, that the latter ionize the gelatin while the bivalent cations will apparently not form ionizable compounds, is the basis of the antagonistic action between the two types of salt. When we treat powdered gelatin with a mixture of NaCl and CaCl<sub>2</sub> the addition of a comparatively small amount of CaCl<sub>2</sub> to M/8 NaCl will inhibit the swelling which would follow if the gelatin were perfused first by the NaCl alone and then by distilled water. It can be shown that when we perfuse 2 gm. of powdered gelatin twice with 25 cc. of 50 cc. M/8 NaCl + 4 cc. M/8 CaCl<sub>2</sub>, and then three times with distilled water, the gelatin is precipitable by alcohol; while if we use the pure M/8 NaCl or 50 cc. M/8 NaCl + 1 or 2 cc. of M/8 CaCl<sub>2</sub> the 3 per cent gelatin solution freed from the salt is not precipitable by alcohol. We, therefore, come to the conclusion that the phenomenon of swelling of gelatin under the influence of neutral salts with univalent cation and the inhibition of this swelling by neutral salts with bivalent cation (alkaline earths) are due to the fact that the former salts cause the formation of ionizable gelatin salts, while the latter apparently cause the formation of non-ionizable gelatin salts.

## II.

We may raise the question: Which of the possible cases of ionization actually occurs when gelatin is treated with neutral salts with univalent cation, like NaCl? If we assume that the gelatin molecule combines with both anion and cation the neutral complex gelatin  $\begin{matrix} \text{Na} \\ \text{Cl} \end{matrix}$  can give rise to three types of protein ions:

- (1) Gelatin-Cl + Na<sup>+</sup>
- (2) Gelatin-Na + Cl<sup>-</sup>
- (3) Gelatin + Na<sup>+</sup> + Cl<sup>-</sup>

In (1) gelatin ion would be negative, in (2) gelatin ion would be positive, and in (3) the gelatin ion would have both a negative and a positive charge in different parts of the molecule. We shall see that the facts speak in favor of (1); namely, an exclusive or a prevailing formation of negative gelatin ions. According to our assumption the additional swelling of gelatin due to ionization should begin when the salt solution used for perfusion to wash out the  $m/8$  NaCl is so dilute as not to suppress the electrolytic dissociation of the gelatin-salt compound completely. Since this suppression depends upon the common ion of the gelatin-salt compound and the salt, it should be possible to ascertain which this common ion is, the anion or the cation of the salt. If, for instance, the dissociation of the gelatin salt occurs according to (1) into gelatin +  $\text{Na}^+$ , the lowest concentration of  $\text{Na}_2\text{SO}_4$  which just allows the additional swelling of a gelatin previously treated with  $m/8$  NaCl should be about one-half of that in which the swelling begins when lower concentrations of NaCl are used; since, *e.g.*, a  $m/256$   $\text{Na}_2\text{SO}_4$  solution sends about twice as many Na ions into solution as does a  $m/256$  NaCl solution. In other words, that concentration of a salt which is just able to inhibit completely the subsequent swelling of gelatin previously treated with  $m/8$  NaCl should depend only upon the concentration of the cation of this salt, if the type (1) of the dissociation of the gelatin salt exists; namely,



This is the case. We know from Table I that the inhibiting concentration of NaCl for the additional swelling<sup>11</sup> of gelatin previously treated with  $m/8$  NaCl is  $m/64$  NaCl. In this concentration an increase of 5 mm. in the height of the cylindrical mass of gelatin will show itself. With a further dilution of NaCl the swelling of the gelatin increases very rapidly.

We now undertook the following series of experiments. We perfused 2 gm. of powdered non-bleached gelatin, as in the pre-

<sup>11</sup> By additional swelling we mean the swelling which takes place as a consequence of the previous treatment with  $m/8$  NaCl, when the excess of the salt is removed and the ionization of the gelatin salt no longer too much depressed.

vious experiments, first with 50 cc.  $M/8$  NaCl, and followed this with a perfusion with solutions of different neutral salts of increasing dilution. Table II gives the results of such a series of experiments.

While the sodium salts with monovalent anion all show the beginning of additional swelling (*i.e.* swelling amounting at least to 5 mm. increase in height of column) in a dilution of  $M/64$  the sodium salts with bivalent anion show this increase in twice this dilution; namely,  $M/128$ . This would indicate that the metal ion alone or prevailingly determines the degree of electrolytic dissociation of the gelatin sodium salt. In other words, the gelatin salt formed, when

TABLE II.

	Additional swelling of 2 gm. powdered gelatin previously perfused twice with 25 cc. $M/8$ NaCl and three times with 25 cc. of the following solutions.											
	$M/8$	$M/16$	$M/32$	$M/64$	$M/128$	$M/256$	$M/512$	$M/1024$	$M/2048$	$M/4096$	$M/8192$	H <sub>2</sub> O
NaCl.....	0	0	2.5	6	15	21	33.5	42	48			54
NaBr.....	0	2.0	2.0	5	12.5	17.5	27	43	42	46	53	52.5
NaNO <sub>3</sub> .....	1	?	4	7	12.5	19.5	29.5	39	43.5	51	49	54
Na acetate.....	1.5	1.5	3	6.5	15	22	31	38	45.5	44	48	55
NaCNS.....		3.5	4	7	12	21	31	40	46	53	58	60
Na <sub>2</sub> SO <sub>4</sub> .....	1	1.5	2.5	3.5	8	14	22.5	30	39	47	55	54
Na <sub>2</sub> oxalate.....	2	2	2	3.5	6.5	14.5	24	29	35	46	50	56
Na <sub>2</sub> tartrate.....	2	2	2	3	5.5	13.5	23	32.5	43	46	52.5	56.5
Na <sub>2</sub> malate.....	0	1	1	2.5	5	13	20	31	35	39	43	51

gelatin is treated with NaCl, dissociates exclusively or prevailingly in the form gelatin + Na<sup>+</sup>. The anion plays obviously a minor if not a negligible part.

When we test 3 per cent solutions of such gelatin with alcohol we find that the solution always becomes non-precipitable as soon as the swelling becomes marked.

When we treat gelatin first with  $M/8$  NaCl and then with dilute solutions of salts of other monovalent cations the results should be essentially the same as if we treated the gelatin with solutions of NaCl, since a compound gelatin-Na should exchange its metal with Li or K or NH<sub>4</sub> without essential molecular alteration. Hence the

results should remain practically the same if a treatment with  $m/8$  NaCl is followed by solutions of NaCl or of LiCl or KCl. This is true, as Table III shows.

For the chlorides the swelling becomes noticeable at a dilution of  $m/64$ , for the sulfates, oxalates, and tartrates at nearly twice the dilution; namely, about  $m/128$ . Only  $\text{Li}_2\text{SO}_4$  is an apparent exception for reasons still to be investigated.

It was shown by special controls that if we first treat powdered gelatin with  $m/8$   $\text{Na}_2\text{SO}_4$  and follow this by weaker concentrations

TABLE III.

	Additional swelling of powdered gelatin perfused twice with 25 cc. m/8 NaCl and three times with 25 cc. of the following solutions.											
	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	m/2048	m/4096	m/8192	H <sub>2</sub> O
LiCl.....	1	1.5	3.5	6	10.5	15	24.5	34.5	44.5	52	53.5	57.5
KCl.....	1	2	2.5	6.5	11.5	16	24	33	38.5	44	46.5	53.5
NH <sub>4</sub> Cl.....	2	2	2	5	13	23	22.5	39.5	48	47		56
Li <sub>2</sub> SO <sub>4</sub> .....	3	0	2	2.5	3.0	10	19.5	30.5	45.5	51	51	60
K <sub>2</sub> SO <sub>4</sub> .....	1	1.5	0.5	3.0	6.5	11	20	29	40.5	42	50.5	54.5
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1.5	1.5	2	3	6	12	18	26	42	50	45	53
K <sub>2</sub> oxalate.....	1	1	1.5	3.5	7.5	17	22.5	25	33.5	43	46.5	55
(NH <sub>4</sub> ) <sub>2</sub> oxalate.....	0	1	1	2.5	5	11.5	22.5	27	36.5	48.5	49	54
K <sub>2</sub> tartrate.....	0	0	1.5	1.5	7	13	19	28	37	42	46	53
(NH <sub>4</sub> ) <sub>2</sub> tartrate.....	2	1.5	2	5 ?	7	16.5	22	35.5	43.5	49.5	52	55

of  $\text{Na}_2\text{SO}_4$  we get the same critical value for the commencement of swelling as when we first treat the gelatin with  $m/8$  NaCl and follow this with weaker concentrations of  $\text{Na}_2\text{SO}_4$ ; namely,  $m/128$ .  $m/8$  LiCl followed by different concentrations of LiCl gives the same critical value for the commencement of swelling as  $m/8$  NaCl followed by different concentrations of LiCl.

If the anion has a distinct effect it must show itself in comparing the effects of washing with  $\text{MgCl}_2$  and  $\text{MgSO}_4$  and of  $\text{CaCl}_2$  and  $\text{CaSO}_4$ . When we perfuse powdered gelatin first with  $m/8$  NaCl and then with  $\text{CaCl}_2$  solutions of a higher degree of dilution we must expect a lower limit of concentration where the swelling begins, since  $\text{CaCl}_2$  by replacing the NaCl in combination with gelatin



transforms the ionizable gelatin into a less ionizable or otherwise modified complex. The concentration of  $\text{CaCl}_2$  must therefore become comparatively low before swelling becomes possible. If the additional swelling, *i.e.* the ionization of the gelatin, depends on the cation alone or predominantly,  $\text{CaSO}_4$  and  $\text{MgSO}_4$  should have the same critical concentration for the commencement of the swelling as  $\text{CaCl}_2$  and  $\text{MgCl}_2$ , which is the case, as Table IV shows. The critical concentration for all the salts is  $m/512$ .

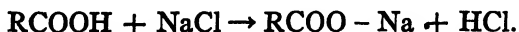
TABLE IV.

Additional swelling of powdered gelatin perfused twice with 25 cc. $m/8$ NaCl and then three times with 25 cc. of the following solutions.												
	$m/8$	$m/16$	$m/32$	$m/64$	$m/128$	$m/256$	$m/512$	$m/1024$	$m/2048$	$m/4096$	$m/8192$	H <sub>2</sub> O
MgCl <sub>2</sub> .....	0	0	0	0	0	4	13	26	42	49	53	52
MgSO <sub>4</sub> .....	1.5	0	0	-1	0	1	7.5	22.5	38	48	55	63
CaCl <sub>2</sub> .....	0	0	0	0	0	1.5	8	24	39	48	51	47
CaSO <sub>4</sub> .....					-3.5	-2.5	+8.5	18.5	37.5	43	47	55
SrCl <sub>2</sub> .....	0	0	0	0	0	1	8	18	36	43	52	52
BaCl <sub>2</sub> .....	0	0	0	0	0	1	10	21	40	45	51	50

All these facts support the idea that neutral salts form compounds with gelatin which dissociate into a positive metal ion (that of the salt used) and into a negatively charged protein ion which may or may not contain the anion of the salt. This idea was tested by experiments on the migration, in an electric field, of gelatin treated with  $m/8$  NaCl and then freed from the salt by washing. Such gelatin migrates to the anode as our theory demands.

### III.

The idea that only the cation enters into the ionization of gelatin might be interpreted to mean that a reaction of the following kind occurs between gelatin and NaCl:



In this case the supernatant solution should have an acid reaction. This was not the case.

Pauli makes another suggestion based on the fact that proteins in general are stronger acids than bases, and that hence they must undergo a stronger hydrolytic dissociation where they act as base than where they act as acid. If such a protein combines with NaCl the Cl would undergo hydrolytic dissociation and be washed away as free HCl. But if this were the case, the water with which NaCl-gelatin is washed should have an acid reaction. Although we were not able to demonstrate such an acid formation, we should remember that little acid was formed at the utmost and that some of it may have been bound again by gelatin molecules. The important fact is that under the influence of a neutral salt, of the type NaCl, the gelatin forms a sodium gelatinate, which dissociates electrolytically into a negatively charged gelatin ion and a positively charged metal ion—that of the salt used.

This assumption is further supported by hydrogen ion determinations which Dr. Dernby has made in my laboratory and which I incorporate here with his permission. The writer had shown in a previous paper that pig's bladder behaves like powdered gelatin inasmuch as it shows a considerable additional swelling in  $H_2O$  when previously treated with NaCl or any neutral salt with univalent cation; while no such additional swelling in  $H_2O$  occurs after previous treatment with neutral salts with bivalent cation. Dr. Dernby found that when the membranes treated with  $m/8$  NaCl (or KCl, etc.) were afterward put into distilled water the latter became slightly alkaline; but that this was not the case when the pig's bladder was previously treated with  $CaCl_2$ .

Dr. Dernby determined the hydrogen ion concentration by Sørensen's colorimetric method, using his standard phosphate solutions and neutral red as indicators. In all these experiments the hydrogen ion concentration changes in the same way as the swelling. Where a strong additional swelling occurs, as in  $H_2O$  after a previous treatment in NaCl, the hydrogen ion concentration diminishes; where no additional swelling occurs no change in the hydrogen ion concentration is observed. In the  $m/8$  NaCl solution where the membrane does not swell no change in the hydrogen ion concentration occurs.

Dr. Dernby's method was as follows. Pieces of pig's bladder of equal weight (about 0.75 gm.) were put into 50 cc. of a salt solution

for 1 hour, then washed repeatedly, and transferred into 50 cc. of distilled water (except in the controls, where the bladder remained permanently in distilled water or in the salt solution).

Table V gives his results.

TABLE V.

After	Hydrogen ion concentration of a solution containing pig's bladder treated as stated (expressed with Sørensen's symbol pH).					
	Control in distilled water.	In M/8 not washed.	In M/8 NaCl washed in H <sub>2</sub> O after 1 hr.	KCl	NH <sub>4</sub> Cl	Na <sub>2</sub> SO <sub>4</sub>
hrs.						
0	About 6.4	6.4	6.4	6.2 to 6.4		
$\frac{1}{2}$	6.4	6.4	6.4			
1	6.4	6.4	6.4	Washed.		
2	7.0	6.4	7.1			
3	7.1	6.6	7.2	6.8	6.8	6.9
5	7.1	6.6	7.3	7.0	7.0	7.0
24	7.1	6.9	7.4	7.3	7.3	7.3

In these experiments the distilled water and salt solutions contained CO<sub>2</sub> and this is the reason why at the first reading the H ion concentration is so high (6.2 to 6.4 instead of 7). The determinations prove that the solutions become more alkaline when the pig's

TABLE VI.

After	Hydrogen ion concentration of a solution containing pig's bladder treated as stated (expressed with Sørensen's symbol pH).			
	Control in distilled water.	In M/8 CaCl <sub>2</sub> 1 hr. then in distilled water.	M/8 MgCl <sub>2</sub>	M/8 SrCl <sub>2</sub>
hrs.				
0	About 6.5	6.4		
$1\frac{1}{2}$		6.4		
		6.4		
		Then transferred into distilled water; immediately after transferring the H ion concentration was in all cases the same pH = 6.2 to 6.4.		
3	6.7	6.4	6.5	6.5
5	7.0	6.6	6.7	6.7
24	7.1	6.9	6.9	6.9

bladder was first treated with a neutral salt with monovalent cation. This agrees with our assumption of an electrolytic dissociation into a negative gelatin ion and a positive  $\text{Na}^+$  ion, since slight hydrolytic dissociation would lead to the formation of a stronger base ( $\text{NaOH}$ ) and a weaker acid, gelatin- $\text{COOH}$ .

We had shown that the bivalent metals,  $\text{Mg}$ ,  $\text{Ca}$ ,  $\text{Sr}$ , and  $\text{Ba}$ , form in all probability less ionizable compounds with gelatin. Hence we should expect that a previous treatment of pig's bladder with  $\text{Ca}$  should not increase the alkalinity of the distilled water. This is actually found to be the case by Dr. Dernby (Table VI).

It is obvious that pig's bladder previously treated with solution of  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{SrCl}_2$  does not cause a change of the same order in the reaction of the water as was found for bladder treated with  $\text{NaCl}$  and  $\text{NH}_4\text{Cl}$ . This harmonizes with the assumption that the bivalent metals form non-dissociable or less dissociable gelatin salts.

#### IV.

In the previous note<sup>8</sup> the writer has stated that the additional swelling caused by a treatment with  $\text{NaCl}$  was observed in powdered gelatin and in pig's bladder, but not in solid blocks of gelatin. It was found that the cause of this difference is the fact that it is easy to ionize large masses of powdered gelatin with a salt and then wash the unnecessary salt solution away, while this is difficult if not impossible with solid blocks of gelatin. The salt solution apparently diffuses slowly in and out of the block. For this reason the following modification of the method was adopted. Powdered gelatin was treated with a  $\text{m}/8$   $\text{NaCl}$  solution in the usual way, and the salt solution was then removed by washing the powdered mass three or four times with distilled water. After all the water had run off the mass of gelatin was poured into a beaker and completely liquefied by heating for about 10 minutes in a water bath of about  $40^\circ$ . This homogeneous liquid mass of gelatin was then put into a bag of collodion and exposed to the draft of an electric fan for about 24 hours, when it had lost most of the water. When such blocks of gelatin, previously treated with  $\text{m}/8$   $\text{NaCl}$  and freed from the  $\text{NaCl}$  solution by repeated washings with  $\text{H}_2\text{O}$ , were put into

H<sub>2</sub>O they showed the same excessive swelling as had been observed in the case of the powdered gelatin; while this excessive swelling was prevented when the block of gelatin previously treated with  $m/8$  NaCl was put back into  $m/8$  NaCl.

The following is an example. Into each of three cylindrical funnels were put 2 gm. of finely powdered Cooper's unbleached gelatin. The gelatin in one funnel (I) was perfused six times in succession with distilled water. The second (II) was perfused twice with 25 cc.  $m/8$  NaCl, then four times with 25 cc. of distilled water; the third (III) was perfused six times with 25 cc.  $m/8$  NaCl. After all the water had run out, the weight of the first mass of gelatin was 34 gm., that of the second, 59.15 gm., and of the third 24.4 gm. The previous treatment with salt increased the swelling from 32 to 57 gm. Incidentally the reader will see that contrary to Hofmeister's statement the gelatin did not swell more when permanently treated with  $m/8$  NaCl than it did when permanently treated with H<sub>2</sub>O, since in the latter case it absorbed 32, in the former only 22 gm.—of which part was NaCl.<sup>12</sup> These three lots of gelatin were then put into colloidion bags and exposed to the fan until their respective masses were 8.4 (I), 7.4 (II), and 6.05 (III) gm. The three masses of gelatin were then liquefied by heating to about 40°C., poured into a flat Petri dish, allowed to gelatinize, and then blocks of equal size were stamped out of each; the respective weights of the three blocks were 7.6 (I), 6.04 (II), and 4.19 (III) gm. I and II were put into distilled water, III was put into  $m/8$  NaCl. After 24 hours the weighing gave the following result:

I	II	III
22.02 gm.	52.8 gm.	12.9 gm.

The gelatin treated first with  $m/8$  NaCl swelled after removal of the salt two and one-half times as much in distilled water as did the gelatin not treated previously with NaCl. We are, therefore, justified in saying that blocks of gelatin behave in the same way as does powdered gelatin if only care is taken that the excess of salt is removed from the block before it is exposed to the distilled

<sup>12</sup> Ash determination showed that the gelatin contained in this case the same proportion of NaCl and water as did the  $m/8$  NaCl solution.

water. If a block of gelatin is first put into  $M/8$  NaCl and then into distilled water, we get different results on account of the slowness of the diffusion of the salt into and out of the block.

In experiments with powdered gelatin we had found that the valence of the cation was much more effective than the valence of the anion. A previous treatment of powdered gelatin with  $M/8$   $CaCl_2$  (or  $MgCl_2$  or  $SrCl_2$  or  $BaCl_2$ ) did not cause the excessive swelling which was caused by  $M/8$  NaCl, and it was found that the addition of a small amount of  $CaCl_2$  to NaCl stopped the after-effect of NaCl upon the subsequent swelling of gelatin in  $H_2O$  (antagonistic salt action). The same is true for blocks of gelatin as the following experiment shows.

2 gm. of powdered gelatin were put into each of three cylindrical funnels. Funnel I was perfused six times with 25 cc.  $H_2O$ ; funnel II twice with 25 cc.  $M/8$   $CaCl_2$  and then four times with 25 cc.  $H_2O$ ; and funnel III six times with 25 cc.  $M/8$   $CaCl_2$ . The next day (after all the water had drained off) the weight of the gelatin liquefied by heating to about  $40^\circ$  was

I	II	III
27.8 gm.	25.8 gm.	24.3 gm.

The  $CaCl_2$  treatment had, therefore, little effect upon the swelling of powdered gelatin. The mass of the three lots was then reduced by evaporating in a collodion sac to 7.6, 7.1, and 7.3 gm. each and blocks of (I) 6.2, (II) 6.0, and (III) 4.7 gm. were cut out. Blocks I and II were put into distilled water, and block III into  $M/8$   $CaCl_2$ . After 41 hours the weight was as follows:

I	II	III
25.4 gm.	24.1 gm.	17.3 gm.

Comparing I and II we notice that the previous treatment with  $CaCl_2$  did not increase the swelling of the block of gelatin.

In the same way all the experiments on antagonistic salt action described in the previous paper<sup>8</sup> could be repeated with solid blocks of gelatin.

These and other experiments allow us to state that the influence of salts upon the swelling of powdered gelatin published in the pre-

vious paper holds good for solid blocks of gelatin also if only care is taken to remove the excess of salt from the block. The results obtained on powdered gelatin can, therefore, be utilized for the theory of swelling of gelatin in general.

## V.

Our results contradict the conclusions which are drawn by many authors from the old experiments of Hofmeister<sup>13</sup> on the influence of neutral salts and sugar on the swelling of gelatin. Hofmeister stated that the salts, according to their effect upon the swelling of gelatin, may be divided into two groups. The one group makes the gelatin swell more than distilled water, the other makes it swell less than distilled water. The former group includes NaBr, NaNO<sub>3</sub>, NH<sub>4</sub>Cl, NaCl, KCl. The second group includes the acetates, citrates, tartrates, and sulfates; but also alcohol, grape sugar, and cane sugar. From these observations it has generally been concluded that the anion is the decisive factor for the swelling; and that Hofmeister's results agree with the influence of salts on other qualities of proteins. Such a conclusion overlooks Hofmeister's distinct statement that alcohol and sugars act like acetates and tartrates. This fact is of importance since it confirmed Hofmeister in his idea that the attraction of the salt for water was one of the factors by which salts and sugars influence the swelling of gelatin. Thus he states that the sulfates tartrates, citrates, and acetates attract water more powerfully than chlorides or bromides and hence prevent the gelatin from absorbing as much water as it does in pure water or in the presence of chlorides.

Hofmeister's method cannot give any clear idea concerning the influence of salts on the swelling of gelatin since this influence depends chiefly upon the ionization of the gelatin. Two processes are necessary to obtain the correct estimate of the effect of this ionization; first, treatment of the whole mass of gelatin with a sufficiently high concentration of the neutral salt ( $M/8$  or  $M/4$ ), and such a treatment can only be effective if the protein is in finely divided condition; and second, removal of the excess of salt in order to per-

<sup>13</sup> Hofmeister, F., *Arch. exp. Path. u. Pharm.*, 1891, xxviii, 210.

mit the electrolytic dissociation of the protein. Neither condition is fulfilled in Hofmeister's method. This also explains why he got similar results with sugar and alcohol as with sodium acetate and sodium sulfate. The differences Hofmeister observed in the action of different salts are comparatively slight and they cannot be used for a theory of the action of salts on swelling.

Lenk<sup>14</sup> tried to demonstrate the antagonistic action between NaCl and CaCl<sub>2</sub> on gelatin. He used Hofmeister's method, trying to show that blocks of gelatin swell less in NaCl solution when CaCl<sub>2</sub> is added, but the effects observed are small and, as the writer believes, within the limits of error of such experiments. Fenn<sup>15</sup> tried to show that gelatin solutions require less alcohol for precipitation in the presence of NaCl when CaCl<sub>2</sub> is added. This is a much more promising method for the study of antagonistic salt action than the one used by Lenk, but the results of Fenn published in his preliminary notices show that he also studied the effects of salts on protein without first removing the excess of salt so that he missed the ionization effect.

### *Theoretical Remarks.*

The paper gives a new and convenient method for the investigation of the effects of electrolytes on the physical properties of proteins and other so called colloids. This method leads to entirely different results from those obtained by the old method of Hofmeister on the swelling of gelatin, and this difference is due to the fact that in Hofmeister's method the effect of the salt on swelling is observed in the presence of an excess of salt, which, as our method shows, inhibits the additional swelling effect of the salt. This latter is the only characteristic effect of the salt on the swelling. Hence it is not possible to discover by Hofmeister's method the true character of the effect of neutral salts (or any true effect of salts) upon the swelling of gelatin.

The most important result obtained with our method is the proof that the influence of salts upon the swelling of gelatin is of a stoichiometrical character; *i.e.*, we can utilize the limiting concentration

<sup>14</sup> Lenk, E., *Biochem. Z.*, 1916, lxxiii, 15, 58.

<sup>15</sup> Fenn, W. O., *Proc. Nat. Acad. Sc.*, 1916, ii, 534, 539.



of different neutral salts for the additional swelling of sodium gelatin to ascertain the molecular concentration of the salt. Experiments on the action of salt upon gelatin treated previously with acid or alkali harmonize with the results given in this paper. They will be discussed in a following paper. The fact that dried pig's bladder behaves similarly to the powdered gelatin indicates the more general character of our results.

In the explanation of these phenomena the writer has adopted the idea that it is the degree of ionization of gelatin salts (of the type sodium gelatin) which determines the additional swelling observed when the gelatin is first treated with a high concentration of an alkali metal salt ( $M/8$  or  $M/4$ ) and the salt has been washed away. The reasons for this assumption are given in the paper and need not be repeated here; but we may add that the new method and the new stoichiometric facts do not depend upon this hypothesis. If we adopt the ionization hypothesis, which seems supported by the facts known at present and contradicted by none, it follows, from our observations that we have at present two cases in which antagonistic salt action is clearly due to the fact that one electrolyte, *e.g.*  $\text{NaCl}$  or acid, causes the formation of ionized protein, while the other electrolyte, *e.g.*  $\text{CaCl}_2$  or neutral salts in general, causes the transformation of ionized into non-ionized protein, or inhibits in some other way the swelling effect of ionization of the protein molecule. The observations on the influence of neutral salts on the diffusion of potassium salts into the egg of *Fundulus* may form a third case.

#### SUMMARY.

1. A new method has been described which allows us to study the effect of neutral salts on gelatin. The essential part of this method consists in using the protein in powdered form, applying the salt in not too low a concentration ( $M/8$  or  $M/4$ ), and then washing away the salt solution.

2. This method has led to the result that the influence of neutral salts on the swelling of gelatin is of a stoichiometric order. Powdered gelatin, when perfused by  $M/8$  or  $M/4$  solution of  $\text{NaCl}$ , shows an additional swelling when afterward perfused by a weaker solu-

tion of a neutral salt with univalent metal. This *additional* swelling is only possible as long as the weaker solution remains below a certain concentration. A comparison of this critical concentration for neutral salts of univalent cations with univalent and with bivalent anions shows that the limiting molecular concentration for the *additional* swelling is twice as great if the anion is univalent as when it is bivalent; regardless of the nature of the anion and cation. The facts can be best explained on the assumption that the inhibiting effect of the salt upon the additional swelling is due to diminution of the degree of electrolytic dissociation of a metal-protein compound. If we make this assumption, which is supported by the facts known at present, our observations lead to the following conclusions.

3. Neutral salts with a univalent cation (in concentrations of  $M/8$  or  $M/4$ ) form highly ionizable salts with gelatin.

4. It seems that these gelatin salts ionize under formation of a positively charged metal ion (that of the salt used) and a negative gelatin ion which may or may not contain the anion of the salt in non-dissociated bondage.

5. The formation of these gelatin ions causes the considerable additional swelling when the gelatin is first treated with the salt and then, after the salt is washed away, is exposed to distilled water.

6. The metals of the alkaline earth group form salts with protein of the type calcium gelatinate, which are not capable of swelling and perhaps little or not at all of ionization. The transformation of protein salts with univalent cation (type sodium gelatinate) capable of swelling into protein salts with bivalent cation (type calcium gelatinate) not capable of swelling is the cause of the antagonistic action of the metals of the calcium group.

7. These results contradict the conclusions drawn from Hofmeister's experiments on the swelling of gelatin and it is pointed out that he was misled by a method not suited for the purpose.



## FURTHER EXPERIMENTS ON THE SEX OF PARTHENOGENETIC FROGS.

By JACQUES LOEB.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Communicated, January 23, 1918.)

It seemed necessary to furnish proof that by the methods of artificial parthenogenesis not only normal larvae can be produced from unfertilized eggs but that these larvae can also develop into full sized normal adults. This task is difficult to accomplish in sea urchins and thus far only Delage has reported that he has succeeded in raising one parthenogenetic larva of a sea urchin to the sexually mature form.

The possibility of producing artificial parthenogenesis in the eggs of the frog by the method of puncture, as demonstrated in the experiments of Guyer and of Bataillon, seemed more promising. The writer has made use of this method for deciding the question whether such frogs can reach the adult size, and for determining their sex. He has now raised twenty leopard frogs to an age of from ten to eighteen months, and nine of these frogs are still alive. Some of these male frogs have reached the full size of the adult male leopard frog. *We are, therefore, entitled to say that the frogs produced by artificial parthenogenesis can develop into adults of full size and of an entirely normal character.*

Loeb and Bancroft<sup>1</sup> tried to ascertain the sex of a parthenogenetic frog immediately after metamorphosis but found the gonads in the intermediate stage, i.e., testes containing a few eggs, though it was obvious that the frog was developing into a male. It was clear that older frogs were needed for the decision of the problem of sex. The writer has been able to ascertain the sex in nine frogs of the age of from ten to eighteen months, and in all of these the ambiguity inherent in the younger frogs had disappeared. He has already reported

<sup>1</sup>Loeb, J., and Bancroft, F. W., *J. Exp. Zool., Wistar Inst., Philadelphia*, 14, 1913, (275); 15, 1913, (379).

that the first two of these parthenogenetic frogs had normal mature testes containing fully developed spermatozoa.<sup>2</sup> No eggs were found in these testes.

The next four frogs examined were also males, so that the problem seemed settled when a year ago last summer one parthenogenetic frog, sixteen months old, was found whose gonads were macroscopically and microscopically well developed ovaries. The next frog was again a male. Although the possibility of an error in method seemed excluded the writer did not wish to publish the fact that both sexes appear in parthenogenetic frogs without having checked the result by a new series of experiments.

These experiments were started in February, 1917. The same precautions as in the older experiments were used. Copulating females which had not yet laid any eggs were separated from their males and kept separated for at least twenty-four hours. The females were repeatedly washed with water during the time of isolation, and directly before the experiment were submerged in 90% alcohol and left there to die. They were taken out, their abdominal cavity was opened with sterilized instruments and the oviduct laid bare. The eggs were taken out from the oviduct with sterilized instruments, and precautions were taken that the eggs did not come in contact with the hands of the experimenter or with the skin or outside of the frog. Alternate lots of about 50 to 100 eggs were punctured or kept untreated as controls. None of these non-treated eggs ever developed. From the punctured, unfertilized eggs ten developed into frogs, of which nine are still alive. The tenth was killed December 21 and the microscopic examination of its gonads showed that it was a female. This leaves then no doubt that both sexes can be produced from the unfertilized eggs of the frog. We have thus far obtained seven male frogs and two females, while the determination in two was missed by accident.

How can we account for the production of both sexes? The diploid number of chromosomes in the frog seems to be 26, according to Swingle,<sup>3</sup> and, therefore, the haploid number 13. The question then

<sup>2</sup> Loeb, J., these PROCEEDINGS, 2, 1916, (313); *The Organism as a Whole*, New York, 1916.

<sup>3</sup> Swingle, W. W., *Biol. Bull., Wood's Hole*, 33, 1917, (70).

arises: Do we find the diploid or haploid number of chromosomes in the cells of the parthenogenetic frog? Brachet<sup>4</sup> found the diploid number in the somatic cells of a parthenogenetic tadpole eighteen days old, but, of course, it was out of the question to ascertain the sex of the tadpole.

The gap can be filled by counting the chromosomes in the fully developed parthenogenetic frogs. Thus far the sections of the testes of only one of the writer's parthenogenetic frogs have been examined cytologically. This male was seventeen months old, had reached the full size of the adult, and had large testes with ripe spermatozoa. Prof. R. Goldschmidt, who was good enough to examine some of the sections, counted over 20 chromosomes, and there can be no doubt that this parthenogenetic male frog possessed the diploid number of chromosomes. The writer has not yet been able to ascertain whether the nuclei of the female frogs have the haploid or diploid number.

It is not known whether the female or male is homozygous for sex in the frog. If the female were homozygous it would mean that the haploid number of chromosomes would be  $12 + x$  and the diploid  $24 + 2x$ . In this case only a female could have the diploid number since  $2x$  would determine a female. Since we find the diploid number of the male parthenogenetic frog the assumption of homozygosity of the female is inadequate if not excluded. If we assume that the female is heterozygous for sex, and that it has the chromosome constitution  $24 + x + y$  (where  $y$  may be missing), the male must have the chromosome constitution  $24 + 2x$ . The haploid number in the egg would be<sup>5</sup>  $12 + x$ , and the diploid number either  $24 + 2x$  or  $24 + x + y$ . The diploid number  $24 + 2x$  would give rise to a male, while a female might be produced by either the haploid number  $12 + x$  or the diploid number  $24 + x + y$ . It is, therefore, of some interest to find out whether or not the female has the haploid number  $12 + x$  chromosomes. It is useless to enter into further speculation until this point is decided, which the writer hopes may be possible in the near future.

<sup>4</sup> Brachet, A., *Arch. Biol., Paris-Bruxelles*, 26, 1911, (362).

<sup>5</sup> The other haploid number  $12 + y$  may be left out of consideration for the present since it is possible that such eggs may not be able to develop.

## SUMMARY.

The author has raised twenty leopard frogs produced by the methods of artificial parthenogenesis from unfertilized eggs to the age of from ten to eighteen months. Nine of these frogs are still alive. Some have reached the size of the full grown normal adult male. Both sexes are represented among the parthenogenetic frogs. Seven of the nine older frogs whose gonads were examined were males, and two were females. The parthenogenetic males possessed the diploid number of chromosomes.

## STUDIES IN ACIDOSIS.

### VII. THE DETERMINATION OF $\beta$ -HYDROXYBUTYRIC ACID, ACETO- ACETIC ACID, AND ACETONE IN URINE.\*

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\* Reported at the Society for Experimental Biology and Medicine, April 11,  
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#### DESCRIPTION OF METHODS.

The methods are based on a combination of Shaffer's oxidation of  $\beta$ -hydroxybutyric acid to acetone and Denigès' precipitation of acetone as a basic mercuric sulfate compound. Oxidation and precipitation are carried out simultaneously in the same solution, so that the technique is simplified to boiling the mixture for an hour and a half under a reflux condenser, and weighing the precipitate which forms. The acetone and acetoacetic acid may be determined either with the  $\beta$ -hydroxybutyric acid or separately. Neither the size of sample nor mode of procedure have required variation for different urines; the same process may be used for the smallest significant amounts of acetone bodies and likewise for the largest that are encountered. The precipitate is crystalline and beautifully adapted to quick drying and accurate weighing; but when facilities for weighing are absent the precipitate can be redissolved in dilute hydrochloric acid and the mercury titrated with potassium iodide by the method of Personne (1863).

Preservatives other than toluene or copper sulfate should not be used.

#### *Solutions Required.*

**20 per cent Copper Sulfate.**—200 gm. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  dissolved in water and made up to 1 liter.

**10 per cent Mercuric Sulfate.**—73 gm. of pure red mercuric oxide dissolved in 1 liter of  $\text{H}_2\text{SO}_4$  of 4 N concentration.

*50 volume per cent Sulfuric Acid.*—500 cc. of sulfuric acid of 1.835 specific gravity, diluted to 1 liter with water. Concentration of  $\text{H}_2\text{SO}_4$  must be readjusted if necessary to make it 17.0 N by titration.

*10 per cent Calcium Hydroxide Suspension.*—Mix 100 gm. of Merck's fine light "reagent"  $\text{Ca}(\text{OH})_2$  with 1 liter of water.

*5 per cent Potassium Dichromate.*—50 gm.  $\text{K}_2\text{Cr}_2\text{O}_7$  dissolved in water and made up to 1 liter.

*Combined Reagents for Total Acetone Body Determination.*—1 liter of the above 50 per cent sulfuric acid, 3.5 liters of the mercuric sulfate, 10 liters of water.

*Removal of Glucose and Other Interfering Substances from Urine.*

Place 25 cc. of urine in a 250 cc. measuring flask. Add 100 cc. of water, 50 cc. of copper sulfate solution, and mix. Then add 50 cc. of 10 per cent calcium hydroxide, shake, and test with litmus. If not alkaline, add more calcium hydroxide. Dilute to the mark and let stand at least one-half hour for glucose to precipitate. Filter through a dry folded filter. This procedure will remove up to 8 per cent of glucose. Urine containing more should be diluted enough to bring the glucose down to 8 per cent. The copper treatment is depended upon to remove interfering substances other than glucose, and should therefore *never be omitted, even when glucose is absent*. The filtrate may be tested for glucose by boiling a little in a test-tube. A precipitate of yellow cuprous oxide will be obtained if the removal has not been complete. A slight precipitate of white calcium salts always forms, but does not interfere with the detection of the yellow cuprous oxide.

*Simultaneous Determination of Total Acetone Bodies (Acetone Acetoacetic, and Hydroxybutyric Acid) in One Operation.*

Place in a 500 cc. Erlenmeyer flask 25 cc. of urine filtrate. Add 100 cc. of water, 10 cc. of 50 per cent sulfuric acid, and 35 cc. of the 10 per cent mercuric sulfate. Or in place of adding the water and reagents separately, add 145 cc. of the "combined reagents." Connect the flask with a reflux condenser having a straight condensing tube of 8 or 10 mm. diameter and heat to boiling. *After boiling*

has begun, add 5 cc. of the 5 per cent dichromate through the condenser tube. Continue boiling gently  $1\frac{1}{2}$  hours. (This time may, if desired, be shortened to 30 minutes by adopting the conditions described on page 337). The yellow precipitate which forms consists of the mercury sulfate-chromate compound (for composition see p. 342) of the preformed acetone, and of the acetone which has been formed by decomposition of acetoacetic acid and by oxidation of the hydroxybutyric acid. It is collected in a Gooch or "medium density" alundum crucible, washed with 200 cc. of cold water, and dried for an hour at  $110^{\circ}$ . The crucible is allowed to cool in room air (a desiccator is unnecessary and undesirable) and weighed. Several precipitates may be collected, one above the other, without cleaning the crucible. As an alternative to weighing, the precipitate may be dissolved and titrated, as described below.

#### *Acetone and Acetoacetic Acid.*

The acetone plus the acetoacetic acid, which completely decomposes into acetone and  $\text{CO}_2$  on heating, is determined without the hydroxybutyric acid exactly as the total acetone bodies, except that (1) no dichromate is added to oxidize the hydroxybutyric acid and (2) the boiling must continue for not less than 30 nor more than 45 minutes. Boiling for more than 45 minutes splits off a little acetone from hydroxybutyric acid even in the absence of chromic acid.

#### *$\beta$ -Hydroxybutyric Acid.*

The hydroxybutyric acid alone is determined exactly as total acetone bodies except that the preformed acetone and that from the acetoacetic acid are first boiled off. To do this the 25 cc. of urine filtrate plus 100 cc. of water are treated with 2 cc. of the 50 percent sulfuric acid and boiled in the open flask for 10 minutes. The volume of solution left in the flask is measured in a cylinder. The solution is returned to the flask, and the cylinder washed with enough water to replace that boiled off and restore the volume of the solution to 127 cc. Then 8 cc. of the 50 per cent sulfuric acid and 35 cc. of mercuric sulfate are added. The flask is connected under the condenser and the determination is continued as described for total acetone bodies.

*Blank Determination of Precipitate from Substances in Urine Other than the Acetone Bodies.*

The 25 cc. aliquot of urine filtrate is treated with sulfuric acid and water and boiled 10 minutes to drive off acetone. The residue is made up to 175 cc. with the same amounts of mercuric sulfate and sulfuric acid used in the above determinations, but without chromate, and is boiled under the reflux for 45 minutes. Longer boiling splits off some acetone from  $\beta$ -hydroxybutyric acid, and must therefore be avoided. The weight of precipitate obtained may be subtracted from that obtained in the above determination.

The blank is so small that in our experience it is relatively significant only when compared with the small amounts of acetone bodies found in normal or nearly normal urines (see p. 350). In routine analyses of diabetic urines we do not determine it.

*Test of Reagents.*

When the complete total acetone bodies determination, including the preliminary copper sulfate treatment, is performed on a sample of distilled water instead of urine no precipitate whatever should be obtained. This test must not be omitted.

*Titration of the Precipitate.*

Instead of weighing the precipitate, one may wash the contents of the Gooch, including the asbestos, into a small beaker with as little water as possible, and add 15 cc. of 1 N HCl. The mixture is then heated, and the precipitate quickly dissolves. In case an alundum crucible is used, it is set into the beaker of acid until the precipitate dissolves, and then washed with suction, the washings being added to the beaker. In place of using either a Gooch or alundum crucible one may when titration is employed, wash the precipitate without suction on a small quantitative filter paper, which is transferred with precipitate to the beaker and broken up with a rod in 15 cc. of 1 N HCl.

In order to obtain a good end-point in the subsequent titration it is necessary to reduce the acidity of the solution. For this pur-

pose we have found addition of excess sodium acetate the most satisfactory means. 6 to 7 cc. of 3 M acetate are added to the cooled solution of redissolved precipitate. Then the 0.2 M KI is run in rapidly from a burette with constant stirring. If more than a small amount of mercury is present, a red precipitate of  $\text{HgI}_2$  at once forms, and redissolves as soon as 2 or 3 cc. of KI in excess of the amount required to form the soluble  $\text{K}_2\text{HgI}_4$  have been added. If only a few mg. of mercury are present, the excess of KI may be added before the  $\text{HgI}_2$  has had time to precipitate, so that the titrated solution remains clear. In this case not less than 5 cc. of the 0.2 M KI are added, as it has been found that the final titration is not satisfactory if less is present. The excess of KI is titrated back by adding 0.05 M  $\text{HgCl}_2$  from another burette until a permanent red precipitate forms. Since the reaction utilized is  $\text{HgCl}_2 + 4 \text{KI} = \text{K}_2\text{HgI}_4 + 2 \text{KCl}$ , 1 cc. of 0.05 M  $\text{HgCl}_2$  is equivalent in the titration to 1 cc. of the 0.2 M KI.

In preparing the two standard solutions the 0.05 M  $\text{HgCl}_2$  is standardized by the sulfide method, and the iodide is standardized by titration against it. A slight error appears to be introduced if the iodide solution is gravimetrically standardized and used for checking the mercury solution, instead of *vice versa*.

In standardizing the mercuric chloride we have found the following procedure convenient: 25 cc. of 0.05 M  $\text{HgCl}_2$  are measured with a calibrated pipette, diluted to about 100 cc., and  $\text{H}_2\text{S}$  is run in until the black precipitate flocculates and leaves a clear solution. The  $\text{HgS}$ , collected in a Gooch crucible and dried at  $110^\circ$ , should weigh 0.2908 gm. if the solution is accurate.

Both by gravimetric analyses of the basic mercuric sulfate-acetone precipitate and by titration, we find the mercury content of the precipitate to average 76.9 per cent. On this basis, each cc. of 0.2 M KI solution, being equivalent to 10.0 mg. of Hg, is equivalent to  $\frac{10.0}{0.769} = 13.0$  mg. of the mercury acetone precipitate.

Titration is not quite so accurate as weighing but, except when the amounts determined are very small, the titration is satisfactory (see p. 345).

*Factors for Calculating Results.*

1 mg. of  $\beta$ -hydroxybutyric acid yields 8.45 mg. of precipitate.

1 mg. of acetone yields 20.0 mg. of precipitate.

1 cc. of 0.2 M KI solution is equivalent to 13 mg. of precipitate in titration of the latter.

*Special Factors for Calculation of Results when 25 Cc. of Urine Filtrate, Equivalent to 2.5 Cc. of Urine, Are Used for the Determination.*

Determination performed.	Acetone bodies, calculated as gm. acetone per liter of urine, indicated by	
	1 gm. of precipitate.	1 cc. of 0.2 M KI solution.
Total acetone bodies.* .....	24.8	0.322
$\beta$ -hydroxybutyric acid .....	26.4	0.344
Acetone plus acetoacetic acid .....	20.0	0.260

\* The "Total acetone bodies" factor is calculated on the assumption that the molecular proportion of them in the form  $\beta$ -hydroxybutyric acid is 75 per cent of the total, which proportion is usually approximated in acetonuria. (See for example, table on pp. 352-353.) Because hydroxybutyric acid yields only 0.75 molecule of acetone, the factors are strictly accurate only when this proportion is present, but the error introduced by the use of the approximate factors is for ordinary purposes not serious. The actual errors in percentages of the amounts determined are as follows: molecular proportion of acetone bodies as  $\beta$ -acid 50, error -6.5 per cent;  $\beta$ -acid 0.60, error -3.8 per cent;  $\beta$ -acid 0.80, error 1.3 per cent.

In order to calculate the acetone bodies as  $\beta$ -hydroxybutyric acid rather than acetone, use the above factors multiplied by the ratio of the molecular weights  $\frac{\beta\text{-acid}}{\text{acetone}} = \frac{104}{58} = 1.793$ . In order to calculate the acetone bodies in terms of molecular concentration, divide the factors in the table by 58. To calculate cc. of 0.1 M acetone bodies per liter of urine, use the above factors multiplied by  $\frac{10,000}{58} = 172.4$ .

## DISCUSSION.

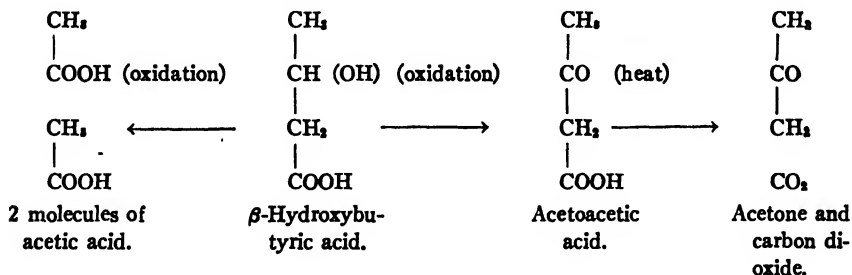
Since the cumulative results of Stadelmann (1883), Külz (1884), Minkowski (1884), and Magnus-Levy (1899) demonstrated the

predominant rôle of  $\beta$ -hydroxybutyric acid in the production of diabetic coma,<sup>1</sup> need for satisfactory means of determining this substance has been recognized. Of the resultant methods those which have proven practical are of two classes, polarimetric and oxidative. In the former the acid is extracted with ether, either directly from the acidified urine (for review of such methods see Hurtley, 1916) or from the powder obtained by mixing it with plaster of Paris (Black, 1908), and the acid, which has a specific rotation of  $-24.12^\circ$ , is estimated by polarizing the extract. In the oxidative methods, which we owe to Shaffer, the hydroxybutyric acid is oxidized to acetone with sulfuric acid and dichromate. The acetone is distilled off during the oxidation and is collected and determined either iodometrically (Shaffer, 1908) or by determining the mercury precipitated by reaction of the acetone with Scott-Wilson's mercuric cyanide reagent (Marriott, 1913, *a*; Kennaway, 1914), or, for the minute amounts obtained in 1 cc. of blood, by estimating the Scott-Wilson precipitate with a nephelometer (Marriott, 1913, *b*; Folin and Denis, 1916).

The extraction-polarimetric method is fairly reliable. The extraction, however, is time-consuming, and the polarization requires for determining the amounts of the substance found in urine the most accurate and expensive type of polariscope. Furthermore, only the  $\beta$ -hydroxybutyric acid is measured, not the acetoacetic acid nor the acetone, although these substances are no less significant of incomplete fat oxidation.

<sup>1</sup>Stadelmann (1883) showed that an organic acid, yielding crotonic acid when distilled with strong sulfuric, is present in the urine in diabetic coma; Minkowski (1884) and Külz (1884) independently identified the organic acid as  $\beta$ -hydroxybutyric; and Magnus-Levy (1899) showed that the amounts present in the body after coma are sufficient to account for fatal acid intoxication. It has also been shown that when  $\beta$ -hydroxybutyric acid is formed in the human organism it is accompanied by acetoacetic acid, usually in an amount not more than one-third that of  $\beta$ -hydroxybutyric acid, as well as by a small amount of acetone, which is presumably formed by decomposition of the unstable acetoacetic acid. Both acids apparently occur in man whenever combustion of fatty acids derived from either fats or amino-acids is incomplete. The excreted  $\beta$ -hydroxybutyric and acetoacetic acids may amount to as much as 36 per cent of the consumed fat (Magnus-Levy, 1905).

Presumably because of these disadvantages of the polarimetric methods, most of the determinations reported in recent years<sup>2</sup> have been made with the methods of oxidation and distillation. The Shaffer technique requires no expensive equipment, and as the acetone and the acetoacetic acid, which decomposes into acetone on boiling, can be obtained by a short preliminary distillation, all three "acetone bodies" can be determined in a single sample of urine, and with a single set of apparatus. The method has been, however, not entirely free from disadvantages. The acetone distilled from the oxidized hydroxybutyric acid is accompanied by impurities which necessitate treating with alkaline peroxide and redistilling before the acetone can be titrated. A more important drawback is the difficulty of maintaining the conditions necessary for constant results. As noted by Shaffer, about 10 per cent of the hydroxybutyric acid is, under the best conditions, decomposed by reactions which yield no acetone. Part of the oxidation breaks the molecule at the  $\beta$ -carbon, where the hydroxyl is attached, and yields other products than acetone, the chief among them being apparently acetic acid (see p. 336).



As a matter of fact, the oxidation may be so conducted that most of the product consists of substances which yield no acetone (see p. 19).

As shown by Shaffer, the yield of acetone is decreased by increase in concentrations of chromic and sulfuric acids. On the other

<sup>2</sup> A noteworthy exception is the paper by Hurtley (1916) in which 48 hour extraction of the urine was used in routine analysis to obtain the hydroxybutyric acid, the acetone plus diacetic being determined by distillation.



hand, the *speed* of oxidation increases with the concentration of these reagents (see pp. 331-332). When all concentrations are being constantly changed by removal of the water distilled away, and by reduction of chromic acid replaced according to the color changes of the mixture, it is difficult to avoid the occasional occurrence of either increased concentration of the reagents, with lowered acetone yield, or decreased concentration, with incomplete oxidation in the allotted time. Presumably because of the difficulty of controlling these factors, the Shaffer determination makes unusual demands on the judgment of the analyst.

It seemed possible that the difficulties might be obviated by precipitating instead of distilling the acetone as it is formed. Denigès, in an investigation of organic compounds of mercury, found that acetone boiled under a reflux with a solution of sulfuric acid and mercuric sulfate forms a crystalline mercury complex approximately twenty times as heavy as the acetone, and of extraordinary insolubility both in dilute sulfuric acid and in water. He recommended it for precipitation of acetone directly in urine, and reported a few determinations. Since then Oppenheimer (1899) and Sammett (1913) have confirmed Denigès in tests on pure acetone solutions, and on normal urines to which acetone was added. Since Denigès' precipitant, like the chromic acid oxidizing agent, acts in sulfuric acid solution and at boiling temperature, it seemed possible to reduce the oxidative determination of hydroxybutyric acid to the simple procedure described in the first paragraph of this paper, eliminating both the labor of twice distilling the acetone and the errors attending fluctuations in the concentrations of the chromic and sulfuric acids. This expectation was realized only after a considerable amount of experimentation.

#### EXPERIMENTAL.

##### *Removal of Glucose.*

The first obstacle encountered was the necessity for complete removal of glucose. Amounts of it such as are encountered in diabetic urines invalidate the determination of either acetone or hydroxybuty-

ric acid (see p. 348). This fact probably explains the failure of Denigès' method for acetone determination to attain use in urine analyses, since a large proportion of the urines in which it is desirable to determine acetone are from patients with diabetes mellitus.

The quantitative removal of glucose was attained by use of the fact, discovered by Salkowski (1879), that glucose forms a complex with copper which is completely precipitated when the solution contains a slight excess of alkali. We find that the optimum alkalinity is attained by saturating the copper-glucose solution with calcium hydroxide. This not only causes complete precipitation of the glucose, but also precipitates all the excess copper as hydroxide; while of the lime itself, but little goes into solution. The precipitate when formed in urine also removes all of the colloidal and coloring matter, as well as some unidentified substances which if not removed form flocculent precipitates subsequently with the mercury reagent. The filtrate obtained from the copper precipitate is water-clear, and contains other than the three acetone bodies only ordinarily negligible traces of substances which yield mercury precipitates under the conditions of the determination. The amount of copper used by Salkowski to precipitate glucose was 5 molecules, one for each hydroxyl of the glucose. We find, however, that a little over half as much completely precipitates the sugar. Precipitation is not instantantaneous, but requires 20 to 30 minutes.

The filtrate may be tested for glucose by merely boiling a few cc. in a test-tube. If any glucose remains in solution it holds some copper with it, and the latter is reduced when the filtrate is boiled. The test is not increased in sensitiveness by adding Fehling's or Benedict's copper solution. A slight white precipitate of calcium salts always forms, as previously mentioned, but this does not obscure the yellow of cuprous oxide when any sugar is present.

*Experiment Showing Time Required for Complete Precipitation of Glucose.—* In each of four 200 cc. flasks were placed 150 cc. of water, in which 1 gm. of Kahlbaum's glucose and 6 gm. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (4.3 molecules) were dissolved. 3 gm. of pulverized calcium hydroxide were added; each mixture was shaken for a minute or two until it became alkaline to litmus, and then diluted to 200 cc. After varying intervals the solutions were passed through folded filters. The sugar in the filtrates was determined by polarizing in 2 dm. tubes. Since

the polariscope could be read to  $\pm 0.01^\circ$ , it sufficed to determine any concentration of glucose exceeding 0.01 per cent. The filtrates were also tested for glucose qualitatively, both by Benedict's method and by merely boiling, as described above, without the addition of any reagent.

Interval between addition of lime and start of filtration.	Rotation of filtrate in 2 dm. tube.	Glucose in filtrate calculated from rotation.	Proportion of total glucose precipitated.	Benedict test for glucose in filtrate.	Reduction test by boiling filtrate alone.
min.	degrees	per cent	per cent		
1	+0.34	0.32	36	+++	+++
5	+0.15	0.14	72	++	++
15	+0.03	0.03	94	+	+
30	0.00	0.00	100	0	0
60	0.00	0.00	100	0	0

*Experiment to Ascertain the Amount of Copper Sulfate Required to precipitate 1 Gm. of Glucose.*—This experiment was performed like the preceding, except that the copper sulfate was varied instead of the time allowed for precipitation, which was constant at 30 minutes. The calcium hydroxide used was proportional to the copper sulfate.

CuSO <sub>4</sub> ·5H <sub>2</sub> O		Calcium hydroxide.	Rotation of filtrate in 2 dm. tube.	Glucose unprecipitated.	Glucose precipitated.	Reduction test in filtrate.
gm.	mols. per 1 mol. glucose	gm.	degrees	gm.	gm.	
2	1.44	1	+0.10	0.200	0.800	+++
4	2.88	2	+0.02	0.040	0.960	+
6	4.33	3	0.00	0.000	1.000	0
8	5.77	4	0.00	0.000	1.000	0

It is evident that 4.33 molecules of crystalline copper sulfate are sufficient to completely precipitate 1 molecule of glucose in half an hour. The fact that 1.44 molecules of copper sulfate precipitate approximately 80 per cent of the glucose indicates that the compound precipitated probably contains only 2 molecules of copper per molecule of glucose, rather than, as Salkowski thought, 1 molecule for each hydroxyl of the sugar.

*Analysis and Rotation of Calcium-Zinc Hydroxybutyrate Used in Experiments.*

The  $\beta$ -hydroxybutyric acid for all our experiments was weighed out in the form of the calcium-zinc salt from a recrystallized and analyzed preparation. The calcium-zinc salt was prepared by Dr. Vinograd-Villchur from diabetic urine by Shaffer and Marriott's method (1913) and recrystallized three times.

• For analysis 0.500 gm. portions were dissolved in 10 cc. of water, and the calcium was precipitated as sulfate by the addition of 5 cc. of 2 N  $\text{H}_2\text{SO}_4$  and 50 cc. of 95 per cent alcohol. The mixture was allowed to stand over night to precipitate. The calcium sulfate was collected in a Gooch crucible and ignited at a low red heat.

The filtrate was concentrated to a few cc. It was then diluted to 150 cc., neutralized with ammonia, and the zinc precipitated with 0.5 gm. ammonium phosphate. The precipitate was dried at  $110^\circ$ .

Substance.	$\text{CaSO}_4$	$\text{ZnNH}_4\text{PO}_4$	Ca	Zn
gm.	gm.	gm.	per cent	per cent
0.5000	0.1314	0.1708	7.72	12.52
0.5000	0.1318	0.1696	7.75	12.43
Calculated for $\text{CaZn}(\text{C}_4\text{H}_7\text{O}_2)_4$ .....			7.74	12.62

Shaffer and Marriott's method for preparing the calcium-zinc salt yields such a beautifully crystalline product that the three recrystallizations were apparently superfluous. The first crop of crystals showed the same rotation as the last. The rotation of the first crop was taken on a solution containing 0.500 gm. of the salt in 20 cc. A 2 dm. tube with a Schmidt and Haensch spectropolarimeter was used.

$$[\alpha]_D^{25} = \frac{-0.77^\circ \times 20}{2 \times 0.500} = -15.4^\circ$$

The third crop gave the following figures: the solution used in this determination was twice as concentrated as the above.

$$[\alpha]_D^{25} = \frac{-1.54^\circ \times 20}{2 \times 1.000} = -15.4^\circ$$

To determine the rotation of the free acid, 0.1506 gm. of the salt, equivalent to 0.1210 gm. of free acid, was dissolved in 2 cc. of  $N$  HCl. The specific gravity of the solution was 1.040.

$$[\alpha]_D^{20} = \frac{-1.35^\circ \times 2.180}{0.1210 \times 1.040} = -23.37^\circ$$

Magnus-Levy (1899) gives  $-24.12$  as the rotation of the pure acid, and Shaffer gives  $-16.2$  as the rotation of the calcium-zinc salt. It is probable that our hydroxybutyric acid was slightly racemized in the process of preparation. We have since found that a gradual loss of rotation occurs when the ether extract of the urine stands, and such extracts yield calcium-zinc salts of low rotation although they give perfect analytical figures for calcium and zinc, and likewise for  $\beta$ -hydroxybutyric acid determined as described in this paper.

*Effect of Changes in Chromic and Sulfuric Acid Concentration:*

Increase in either chromic or sulfuric acid concentration accelerates the oxidation, which by varying these concentrations can be made to run to completion in periods varied at will from 20 minutes to as many hours. Shortening of the reaction period by increasing sulfuric or chromic acid is attained, however, at the cost of a lowered acetone yield, since increase in either reagent increases the proportion of non-acetone products yielded by the oxidation. We are consequently forced to compromise between high yield and quick reaction time. Under the conditions upon which we finally settled as a routine, each molecule of  $\beta$ -hydroxybutyric acid yields 0.75 molecule of acetone, and the entire time required for both oxidation and precipitation of the mercury-acetone complex is 90 minutes. The conditions have been so studied, however, that any one may change them, completing the reaction in 40 minutes, for example, with 60 per cent yield. In the other direction one can, of course, increase the yield by diluting the chromate at will, so long as enough is present to complete the oxidation. The sulfuric acid, however, cannot be very much more dilute than under our standard conditions or basic mercuric sulfate will be precipitated along with the acetone complex.

*Experiment Showing Effect of Sulfuric Acid Concentration on the Velocity and Acetone Yield of the  $\beta$ -Hydroxybutyric Acid Oxidation.*—Three sets of solutions were made up as follows. In each of a series of fifteen 500 cc. Erlenmeyer flasks were placed the following: 20 cc. of the 10 per cent  $\text{HgSO}_4$  solution described at the beginning of this paper; 20 cc. of a solution containing per cc. 1.244 mg. of calcium-zinc hydroxybutyric, equivalent to 1 mg. of the free acid; an amount of 17 N sulfuric acid indicated in the table, and enough water to bring the total volume to 170 cc. The flasks were connected with reflux condensers and heated. After boiling had begun 5 cc. of 5 per cent  $\text{K}_2\text{Cr}_2\text{O}_7$  solution were added to each solution through the reflux condenser. At varying time intervals measured from the moment when the dichromate was added the flasks were removed and cooled quickly under tap water. The precipitates were washed and weighed as usual. The results are given in the following table and in Fig. 1.

17 N $\text{H}_2\text{SO}_4$ added.	Concentration of $\text{H}_2\text{SO}_4$ in total solution.	Weight of precipitate formed from 20 mg. of $\beta$ -hydroxybutyric acid in time indicated.				
		30 min.	60 min.	90 min.	120 min.	150 min.
cc.	N	gm.	gm.	gm.	gm.	gm.
2	0.58	0.0982	0.1433	0.1676	0.1830	0.1884
8	1.17	0.1380	0.1650	0.1660	0.1708	0.1692
16	1.94	0.1314	0.1374	0.1356	0.1364	0.1354

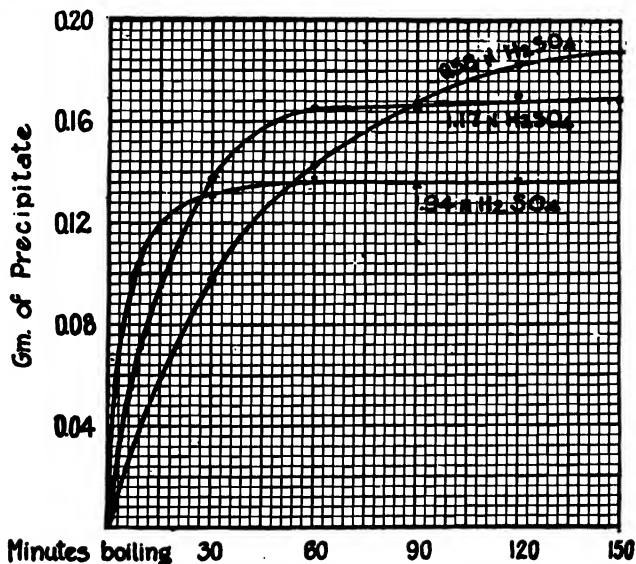


FIG. 1.

*Experiment Showing the Effect of Chromic Acid Concentration on the Velocity and Acetone Yield of the  $\beta$ -Hydroxybutyric Acid Oxidation.*—Each solution contained 10 mg. of  $\beta$ -hydroxybutyric acid. The conditions were those described at the beginning of this paper for determination of "Total acetone bodies" except for variations in the volume of 10 per cent chromate solution added. The volume of the total solution was in each case 175 cc. after the chromate solution had been added. The results are given in the following table and in Fig. 2.

$K_2Cr_2O_7$ gm.	Precipitate formed from 10 mg. $\beta$ -hydroxybutyric acid after time indicated.				
	15 min.	30 min.	60 min.	90 min.	120 min.
0.15	0.0504	0.0752	0.0848	0.0884	0.0906
0.30	0.0652	0.0796	0.0846	0.0861	0.0865
1.00	0.0546	0.0662	0.0656	0.0660	—

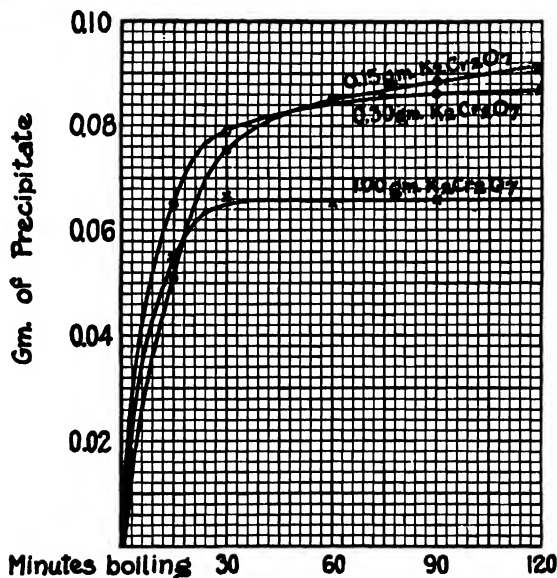


FIG. 2.

*Effect of Mercuric Sulfate Concentration on Yield of Precipitate from  $\beta$ -Hydroxybutyric Acid.*

Each flask contained 175 cc. of solution with 0.25 gm. of potassium dichromate and free sulfuric acid in sufficient amount to bring its concentration to 1.70 N,

Concentration of $\text{HgSO}_4$ .		Yield of precipitate from 10 mg. $\beta$ -hydroxybutyric acid.	
		After 1 hr. boiling.	After 2 hrs. boiling.
gm. per 100 cc.	gm. mols. per liter	gm.	gm.
1	0.034	0.0638	0.0674
2	0.068	0.0756	0.0772
3	0.101	0.0828	0.0832
4	0.133	0.0882	0.1036
5	0.169	0.0974	0.1492
6	0.203	0.1278	

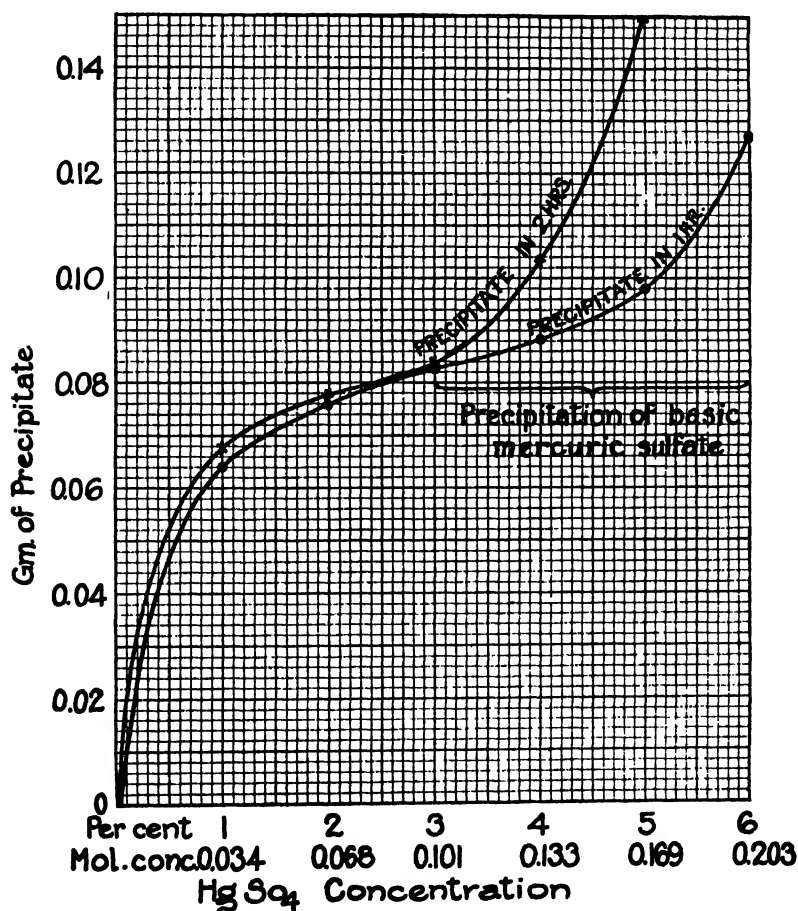


FIG. 3.



or 0.85 M (approximately 1 cc. of concentrated sulfuric to 20 cc. of solution). The mercuric sulfate was varied.

The precipitates obtained with concentrations of mercuric sulfate greater than 3 per cent were flocculent and abnormal in appearance. This was due to contamination with the yellow basic mercuric sulfate which is precipitated when mercury sulfate solutions are heated without sufficient free sulfuric acid to hold all the mercury in solution. The point at which the precipitation of the basic salt begins is shown by the sharp change in direction of the curves of Fig. 3 as the 3 per cent point is passed. Up to this point the curves are concave, approaching a horizontal position at a level which evidently indicates the maximum yield of normally composed mercury-acetone precipitate. Beyond this point, however, the curves suddenly shoot upwards, in a manner explainable only by the formation of a new precipitate having no relation to the acetone present.

Control experiments with only sulfuric acid and mercury sulfate confirmed this explanation. With a greater  $\frac{\text{HgSO}_4}{\text{H}_2\text{SO}_4}$  ratio than that prevailing at the 3 per cent experiment (approximately 1:8 in molecular proportions) precipitation of the basic mercuric sulfate occurs, with or without the presence of acetone. If the  $\text{H}_2\text{SO}_4$  concentration is increased, the  $\text{HgSO}_4$  can also be raised. The yield of precipitate from  $\beta$ -hydroxybutyric acid is diminished rather than increased, however, because of the previously discussed effect of sulfuric acid in diminishing the acetone yield. Thus, with 2.5 instead of 1.7 N  $\text{H}_2\text{SO}_4$  the mercuric sulfate concentration can be raised to 5 per cent without precipitation of basic sulfate, but the yield of acetone precipitate is 10 per cent less than in the above experiment.

*Influence of Temperature on the Yield of Acetone from  $\beta$ -Hydroxybutyric Acid.*

In preliminary experiments to determine the yields of precipitate from varying amounts of  $\beta$ -acid it was found that in a given set of determinations run simultaneously with from 1 to 50 mg. of  $\beta$ -hydroxybutyric acid the yield of precipitate per mg. of acid was constant. Different sets of determinations run on different days, however, gave results which varied considerably, and we were for

a time at a loss to explain this. The clue was finally given by two series, for both of which the solutions, including the dichromate, were mixed early one morning. One series was boiled in the morning and gave an average of 8.0 mg. of precipitate per mg. of acid. The other stood at room temperature until afternoon before it was boiled. In this series the yield of precipitate per mg. of hydroxybutyric acid was 7.2 mg. It appeared possible that in the solution standing the longer time at room temperature a partial oxidation of the  $\beta$ -acid had occurred, and that at the low temperature the reaction took chiefly the path yielding products other than acetone. The following experiment shows that this explanation was correct.

The solutions were made up with reagents in the proportions described at the beginning of this paper for the routine method for "Total acetone bodies." The dichromate, however, was added *before* the solutions were heated, and allowed to act for varying periods at room temperature. The solutions were finally boiled under reflux for 1 hour, so that the intact  $\beta$ -acid would be oxidized in the usual manner. The amount of  $\beta$ -acid present was 18.04 mg. The following results were obtained:

Period of preliminary oxidation at room temperature.	Precipitate yielded on subsequent boiling for 1 hour.	
	From 18.04 mg. $\beta$ -acid.	Per gm. $\beta$ -acid.
	gm.	gm.
0	0.1480	8.21
(Dichromate added through reflux to boiling solution.)	0.1482	8.22
Dichromate added just before heating began.	0.1432	7.94
	0.1432	7.94
3 hours.	0.1274	7.06
	0.1270	7.04
20 "	0.0374	2.07
	0.0384	2.13

In 20 hours at room temperature three-fourths of the  $\beta$ -acid is destroyed by reactions which yield no acetone. Even when heating was begun immediately after the dichromate was added the portion of the oxidation that occurred before boiling temperature was reached reduced the acetone yield by 3 per cent. It was evi-

dent that low results must be encountered when any of the oxidation occurs at temperature below the boiling point. After this was discovered the routine precaution was introduced of adding the dichromate through the reflux condenser after boiling had begun, and the inconsistencies between different sets of determinations disappeared.

*The Volatile Fatty Acid Formed by Oxidation of  $\beta$ -Hydroxybutyric Acid.*

As pointed out on page 325, the structure of  $\beta$ -hydroxybutyric acid leads one to expect it to break partly in the middle on oxidation, yielding two molecules of acetic acid.

In order to obtain the volatile acid for analysis 0.5 gm. of the calcium-zinc salt was mixed with 170 cc. of water containing 20 cc. of the 50 volume per cent sulfuric acid and 20 cc. of 5 per cent potassium dichromate. The mixture was allowed to stand 48 hours in the cold. All the conditions, high sulfuric and chromic acid concentration and low temperature, tend to a low yield of acetone and high yield of the non-acetone products.

The solution was concentrated under diminished pressure to about 50 cc. and the distillate titrated with phenolphthalein as indicator. It was sulfate-free and neutralized 37.7 cc. of 0.1 N sodium hydroxide. The total  $\beta$ -hydroxybutyric acid present at the start would have neutralized 38.6 cc. The yield of volatile acid was therefore one molecule from one molecule of  $\beta$ -hydroxybutyric acid.

The distillate, after being neutralized by the titration, was concentrated to about 20 cc., poured into 80 cc. of absolute alcohol, and 0.5 gm. of silver nitrate in alcoholic solution was added. The white precipitate which formed was washed in a centrifuge with alcohol and ether. It weighed 0.35 gm., and gave the following figures on analysis.

0.3012 gm. of substance gave 0.2017 gm. Ag.

	Calculated for AgC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> :	Found:
Ag.....	64.7	66.9
	Calculated for AgCHO <sub>2</sub> :	
Ag.....	77.1	

The precipitate darkened quickly when exposed to light. This behavior and the analysis indicate that it was silver acetate, accompanied by a smaller amount of formate.

*Experiment Illustrating Conditions for Determination of  $\beta$ -Hydroxybutyric Acid in 30 Minutes.*

Oxidation and precipitation are accomplished most quickly with a high concentration of sulfuric acid and low mercuric sulfate. Solutions were made up as follows:

Volume.....	175 cc.
H <sub>2</sub> SO <sub>4</sub> .....	1.91 N
HgSO <sub>4</sub> .....	2.00 gm. = 1.14 per cent
$\beta$ -Hydroxybutyric acid.....	0.020 gm.
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> .....	0.250 gm.

The dichromate (5 cc. of 5 per cent solution) was added through the reflux condenser in each case after boiling had begun. The flasks were removed from the flame at intervals and cooled under the tap. The yields of precipitate are shown below.

Time of boiling.	Yield of precipitate.	
	From 20 mg. $\beta$ -acid.	Per gm. $\beta$ -acid.
<i>hrs.</i>	<i>gm.</i>	<i>gm.</i>
0.5	0.1318	6.59
1.0	0.1310	6.55
1.5	0.1322	6.61
2.0	0.1318	6.59
3.0	0.1298	6.49

Both oxidation and precipitation were complete in 30 minutes. Since precipitation of acetone requires at least 15 minutes, it is evident that the oxidation itself must have been finished in about 15 minutes. The yield per gm. of  $\beta$ -acid, however, is only 6.6 gm., indicating the formation of but 0.60 molecule of acetone from each molecule of  $\beta$ -acid. Under the conditions chosen for routine work and described in the first part of this paper (2 per cent HgSO<sub>4</sub>, 165 N H<sub>2</sub>SO<sub>4</sub>) 1½ hours of boiling are required, as shown below, to finish the precipitation, but the yield is 0.75 molecule of acetone.

*Time Required for Complete Oxidation and Precipitation under Conditions Chosen for Routine Determination.*

20 cc. portions of a solution containing 1.122 mg. of calcium-zinc hydroxybutyrate, or 0.903 mg. of hydroxybutyric acid, per cc. were

treated in the manner prescribed for urine filtrate in "Determination of total acetone bodies" at the beginning of this paper. Only the period of boiling was varied as indicated in the following table.

Time of boiling.	Yield of precipitate.	
	From 18.06 mg. of $\beta$ -hydroxybutyric acid.	Per gm. of $\beta$ -hydroxybutyric acid.
<i>hrs.</i>	<i>gm.</i>	<i>gm.</i>
1	0.1478	8.19
	0.1464	8.11
1.5	0.1518	8.41
	0.1506	8.35
2.0	0.1512	8.39
	0.1508	8.36

It is evident that both oxidation and precipitation are complete in 1.5 hours. In 1 hour the precipitate is 98 per cent of that obtained after 1.5 or more hours.

#### *The Effect of Filtering Hot versus Filtering Cold.*

Determinations were performed with solutions made up exactly as in the above experiment. The boiling was continued 1.5 hours in all cases. In one pair of determinations the precipitate was filtered immediately after the flame was turned out, while the solution was still boiling hot. In the other pair the solution was allowed to come to room temperature before it was filtered. In both cases cold wash water was used.

#### *Yield of Precipitate from 18.12 Mg. of $\beta$ -Hydroxybutyric Acid.*

Filtered hot.	Filtered after cooling.
<i>gm.</i>	<i>gm.</i>
0.1494	0.1512
0.1502	0.1510
Average.....0.1498	0.1511

It appears that the hot liquid holds in solution about 1 mg. of mercury-acetone complex which adds itself to the precipitate when the mixture is cooled before filtering. The difference is so slight that it may in ordinary analyses be disregarded, and the precipitate filtered either hot or cold according to convenience.

*Yield of Precipitate from Varying Amounts of  $\beta$ -Hydroxybutyric Acid.*

*Under the conditions chosen* for routine determinations the yield of precipitate is approximately 8.45 gm. per 1 gm. of  $\beta$ -hydroxybutyric acid, and the ratio is not affected by any variations in the amount of  $\beta$ -acid present that are within the maximum amounts encountered in urine analyses.

$\beta$ -hydroxybutyric acid.		Yield of precipitate.	
Present.	Corresponding to amount in urine.	Total.	Per 1 gm. $\beta$ -hydroxybutyric acid.
gm.	per cent	gm.	gm.
0.001	0.04	0.0084	8.4
		0.0084	8.4
		0.0088	8.8
		0.0086	8.6
0.005	0.20	0.0422	8.44
		0.0424	8.48
0.010	0.40	0.0844	8.44
		0.0834	8.34
		0.0850	8.50
		0.0835	8.35
0.050	2.00	0.4336	8.42
		0.4364	8.45
		0.4396	8.49
		0.4374	8.47
0.100	4.00	0.9060	9.06
		0.8950	8.95

For amounts of  $\beta$ -hydroxybutyric acid equal to those which would be encountered in urines containing up to 2 per cent of the acid, the factor is constant at approximately 8.45 gm. of precipitate per

gm. of acid. When 4 per cent of  $\beta$ -acid is present the factor is increased to 9.0; but since more than 2 per cent has, so far as we know, never been reported for a human urine, the factor 8.45 may be taken for all urine analyses.

With one mercury solution, made up from one of the cheaper brands of mercuric sulfate, the  $\beta$ -hydroxybutyric acid factor was consistently lower, only 7.6 gm. of precipitate per 1 gm. of  $\beta$ -acid. The factor for pure acetone was not affected. Apparently the mercuric sulfate contained an unidentified impurity which affected the course of the oxidation. All the brands of red mercuric oxide which we were able to obtain gave consistent results, agreeing with those obtained with Merck's "reagent" mercuric sulfate, and consequently we have specified red mercuric oxide rather than mercuric sulfate in the directions for making up the mercury solution.

*Time Required for Complete Precipitation of Acetone.*

Solutions of acetone were treated as described for urine filtrates at the beginning of this paper, under "Determination of acetone and acetoacetic acid" except that the time of boiling was varied. The amount of acetone present was approximately 10 mg. (10 cc. of a 0.1 per cent solution made up by weight from Kahlbaum's "acetone from the bisulfite compound"). The flasks were cooled under the tap as quickly as possible after the period of boiling was over.

Time of boiling.	Yield of precipitate.
<i>min.</i>	<i>gm.</i>
5	0.1776
15	0.1940 0.1958
30	0.1962 0.1996
60	0.1960 0.1976

*Yield of Precipitate from Varying Amounts of Acetone.*

This experiment was conducted like the preceding, except that the time of boiling was kept constant at 30 minutes, the amounts of acetone being varied. Kahlbaum's "acetone from the bisulfite compound" was dried with fused calcium chloride and redistilled, the first and last fractions being rejected and the middle fraction used for the following determination.

0.9670 gm. of this acetone was weighed out into a stoppered flask containing 50 cc. of water. After mixing, the solution thus obtained was diluted to 2 liters, so that 1 cc. contained 0.4835 mg. of acetone. 20 cc. of this solution were diluted to 200, and portions of the diluted solution used for the determinations of acetone amounts below 2 mg. The determinations were carried out as described for "Acetone and acetoacetic acid" at the beginning of this paper.

*Yields of Precipitate from Varying Amounts of Acetone.*

Acetone.	Precipitate.	Average wt. of precipitate.	Precipitate per 1 mg. acetone.
mg.	mg.	mg.	mg.
0.242	4.4 4.8	4.6	19.0
0.484	9.6 9.6	9.6	19.8
0.967	20.0 19.6	19.8	20.5
1.934	40.4 40.0	40.2	20.7
2.901	57.6 58.6	58.1	20.0
3.868	78.2 78.0	78.1	20.2
4.835	96.0 94.8	95.4	19.74
9.670	189.8 191.2	190.5	19.70
19.31	377.5 377.9	377.7	19.55



Under the condition for the determination of acetone and acetoacetic acid the yield of precipitate is, within the limits of analytical error, 20 mg. It falls slightly below 20 with the larger amounts, but for urine and blood analysis, where 150 mg. is the maximum precipitate from acetone and acetoacetic acid, the factor 20 may be taken throughout without causing significant error.

*Composition of the Precipitates Obtained from  $\beta$ -Hydroxybutyric Acid, and from Acetone in Absence and Presence of Chromic Acid.*

50 cc. portions of solutions containing approximately 1 mg. of  $\beta$ -hydroxybutyric acid or 0.5 mg. of acetone per cc. were precipitated as described, for the determinations in urine filtrates, of  $\beta$ -hydroxybutyric acid, total acetone bodies, and acetoacetic acid. The precipitates were weighed in Gooch crucibles, then transferred with the asbestos to beakers, where they were dissolved in 20 cc. portions of normal hydrochloric acid. The asbestos was filtered out, the washings diluting the solutions to about 100 cc. The mercury was precipitated as sulfide and dried to constant weight at  $110^\circ$  in Gooch crucibles. The filtrate from the sulfide was boiled free of  $\text{H}_2\text{S}$ , and the  $\text{SO}_4$  was precipitated by slow addition of 10 cc. of 5 per cent barium chloride to the hot solution. The  $\text{BaSO}_4$  was ignited and weighed in Gooch crucibles. The filtrate from the barium sulfate was freed from barium by addition of sulfuric acid, and any chromate which might have escaped reduction by  $\text{H}_2\text{S}$  was reduced by boiling with alcohol. The chromium was then precipitated as hydroxide with ammonia, was ignited to  $\text{Cr}_2\text{O}_3$ , and weighed in platinum crucibles. The results are given in the accompanying table.

It is evident that, when chromic acid is present in the concentrations used, mercuric chromate or dichromate replaces about one-fourth the mercuric sulfate in the precipitate. The mercury content of the precipitate is but little affected, ranging from 76.6 to 77.0 per cent, whether or not chromate is present and whether the precipitate arises from preformed acetone or from acetone from hydroxybutyric acid.

The composition in the absence of chromate is most nearly indicated by the formula  $3\text{HgSO}_4 \cdot 5\text{HgO} \cdot 0.2(\text{CH}_3)_2\text{CO}$ , which agrees closely with the observed figures for  $\text{SO}_4$  and Hg but indicates 5.5 per cent of acetone instead of the 5.0 per cent (shown by the 1:20 ratio of acetone to precipitate) which is the maximum that can be present if all the acetone is precipitated and none destroyed. Denigès (1898)

gave  $2\text{HgSO}_4 \cdot 3\text{HgO} \cdot (\text{CH}_3)_2\text{CO}$ , which, however, indicates 14.8 per cent  $\text{SO}_4$ , instead of the 13.71 to 13.97 which we find, and only 4.6 per cent of acetone. It appears that the composition varies somewhat according to the conditions under which the precipitate is formed.

*Composition of Precipitates.*

Precipitates from 50 cc. of $\beta$ -hydroxybutyric acid solution boiled with chromic acid as in determination of $\beta$ -hydroxybutyric acid.				Precipitates from 50 cc. acetone solution boiled in presence of chromic acid, as in determination of total acetone bodies.			Precipitates from 50 cc. acetone solution boiled without chromic acid, as in determination of acetone plus acetoacetic acid.		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Mercury-acetone precipitate.....	0.4532	0.4528	0.4510	0.4884	0.4858	0.4870	0.4776	0.4766	0.4742
HgS.....	0.4052	0.4044	0.4010	0.4316	0.4316	0.4373	0.4246	0.4232	0.4198
BaSO <sub>4</sub> .....	0.1184	0.1186	0.1178	0.1184	0.1184	0.1186	0.1621	0.1598	0.1600
Cr <sub>2</sub> O <sub>3</sub> .....	0.0178	0.0186	0.0175	0.0215	0.0217	—			
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Hg.....	77.10	77.01	76.60	76.10	76.58	76.58	76.60	76.58	76.30
SO <sub>4</sub> .....	10.76	10.78	10.75	9.97	10.02	9.94	13.97	13.71	13.88
CrO <sub>4</sub> .....	5.98	6.27	5.92	6.78	6.81	—	0	0	0

*Molecular Proportions.*

	Average of three determinations.		
Hg.....	8.00	8.00	8.00
SO <sub>4</sub> .....	2.35	2.18	3.02
CrO <sub>4</sub> .....	1.09	1.23	0
CrO <sub>4</sub> + SO <sub>4</sub> .....	3.44	3.41	3.02
Cr <sub>2</sub> O <sub>7</sub> + SO <sub>4</sub> .....	2.90	2.80	3.02

When chromate enters the composition it is possible, because of the well known tendency of  $\text{CrO}_4$  salts to form isomorphous crystals with  $\text{SO}_4$  salts, that the chromium is in the form  $\text{CrO}_4$  rather than  $\text{Cr}_2\text{O}_7$ . It is impossible to decide the point from the analytical figures, however. If the Cr is calculated as  $\text{CrO}_4$ , the combining power of  $\text{CrO}_4 + \text{SO}_4$  is greater (3.4 molecules) than that of  $\text{SO}_4$  alone (3.0 molecules) when no chromate is present.

The absolute amount of Hg, as well as of the total precipitate yielded by a given amount of acetone, is also increased by the pres-

ence of chromate (average precipitate = 0.487 gm. with chromate, 0.476 without; HgS yields being 0.431 and 0.422 gm. respectively from these precipitates). It therefore appears that the  $\text{HgCrO}_4$  or  $\text{HgCr}_2\text{O}_7$  not only replaces about one-fourth of the  $\text{HgSO}_4$  in the acetone precipitate, but is also added onto the precipitate sufficient to increase by about 1 part in 40 the weight of the precipitate yielded by a given amount of acetone.

As the result of this fact it would be logical in the calculation of the results of "Total acetone body" determinations, in which chromate is used, to employ the factor 20.5 rather than 20 in estimating the results. It is a matter of empirical observation, however, that when acetone and  $\beta$ -hydroxybutyric acid are thus determined together, there seems to be a slight compensating error, so that the precipitate does not exceed by more than the limit of experimental error the sum of the precipitates obtained in determination of the two substances separately (see table on p. 352). We have therefore used the factor 20 throughout for calculating acetone.

#### *Titration of Mercury in the Precipitates.*

The mercury-acetone precipitate can be readily dissolved in warm dilute hydrochloric acid and estimated by titration of the mercury, since, as shown above, the mercury content is approximately constant at 76.6 to 77.0 per cent. Denigès (1898) himself titrated by adding to the mercuric salt solution an excess of 0.1 N KCN, and titrating with 0.1 N  $\text{AgNO}_3$  the cyanide in excess of the amount required to form  $\text{Hg}(\text{CN})_2$ . Sammett (1913) did not get satisfactory results with the method. We have tried the Volhard titration of the mercury with sulfocyanate. Although it gave excellent results with pure mercuric sulfate solution, it was not even approximately accurate with the redissolved acetone precipitate. We have, however, like Willaman (1916), obtained good results with the old method of Personne (1863).

The following results obtained with the technique already described on pages 321-322, indicate the degree of accuracy of the titration.

*Effect on  $\beta$ -Hydroxybutyric Acid of the Reagents Used in Determining Acetone and Acetoacetic Acid.*

Even without chromic acid,  $\beta$ -hydroxybutyric acid when subjected to prolonged boiling with sulfuric acid and mercuric sulfate splits off a little acetone and yields a weighable precipitate. The amount formed from pure  $\beta$ -acid however is not measurable if the time of boiling is kept below 45 minutes. Nor does presence of the

*Results Obtained by Titration of the Mercury in the Acetone Precipitates.*

$\beta$ -hydroxybutyric acid.	Wt. of precipitate.	0.2 M KI.	0.05 M HgCl <sub>2</sub> .	Excess 0.2 M KI.	$\beta$ -hydroxybutyric acid calculated from titration (excess KI $\times$ 1.538).	Amount of precipitate calculated from titration (excess KI $\times$ 13.0)
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Standard solutions of calcium-zinc salt of  $\beta$ -hydroxybutyric acid.

mg.	mg.	cc.	cc.	cc.	mg.	mg.
2	—	5.00	3.70	1.30	2.00	—
2	—	5.00	3.65	1.35	2.07	—
2	—	5.00	3.70	1.30	2.00	—
10	86.2	8.00	1.45	6.55	10.07	85.2
10	86.0	8.00	1.50	6.50	10.00	84.5
20	171.4	15.00	2.00	13.00	20.00	169.0
20	169.2	15.00	2.25	12.75	19.61	164.8
30	—	21.00	1.15	19.85	30.53	—
30	—	21.00	1.30	19.70	30.24	—
50	—	35.00	1.90	33.10	50.91	—
50	—	35.00	1.75	33.25	51.13	—

Precipitates from total acetone body determinations in urines.

—	69.6	8.00	2.85	5.15	—	66.9
—	67.6	8.00	2.90	5.10	—	66.3
—	70.8	8.00	2.75	5.25	—	68.3
—	138.0	12.50	2.35	10.15	—	132.0
—	140.4	12.50	1.80	10.70	—	139.1
—	678.0	55.00	2.90	52.10	—	677.5
—	645.0	55.00	5.40	49.70	—	646.0

$\beta$ -acid appreciably affect the results of the acetone determination if in the latter the period of boiling is kept under 45 minutes.

*Experiment.*—250 cc. of water, 0.500 gm. of calcium-zinc hydroxybutyrate, 100 cc. of the 20 per cent CuSO<sub>4</sub>·5H<sub>2</sub>O solution, and 10 gm. of calcium hydroxide

were made up to 500 cc. and filtered, as in the preparation of urine for analysis. Of the filtrate 25 cc. portions were treated in Nos. 1 and 2 as described at the beginning of this paper for "Acetone plus acetoacetic acid" and " $\beta$ -hydroxybutyric acid" determinations, the only variation in technique being in the duration of the boiling period for the acetone plus acetoacetic acid determination. In Nos. 3 and 4, 10 cc. portions of 0.102 per cent acetone were added, while 5 and 6 serve as control analyses of the acetone alone.

No.	Acetone present.	$\beta$ -acid present.	Yield of precipitate.					
			Acetone determination (no dichromate).				$\beta$ -hydroxybutyric acid determination (boiled 90 min. with dichromate).	
			Boiled 45 min.		Boiled 90 min.		Precipitate.	$\beta$ -acid calculated from precipitate.
			Precipitate.	Acetone calculated from precipitate.	Precipitate.	Acetone calculated from precipitate.		
	mg.	mg.	gm.	mg.	gm.	mg.	gm.	mg.
1	0	20.1	0.0000	0	0.0032	1.62	0.1684	19.9
2	0	20.1	0.0002	0	0.0048	2.43	0.1720	20.3
3	10.2	20.1	0.1998	9.99				
4	10.2	20.1	0.2036	10.18				
5	10.2	0	0.2022	10.11				
6	10.2	0	0.2012	10.06				

*Determination of  $\beta$ -Hydroxybutyric Acid by Heating at 100° in Pressure Bottles. (Alternative to Boiling under Reflux.)*

Oppenheimer (1899) and Sammett (1913) found that acetone could be determined by heating with the mercury reagent in a pressure bottle immersed in a water bath as well as by Denigès' original method of boiling under a reflux.  $\beta$ -hydroxybutyric acid also may be determined by carrying out the oxidation and precipitation in a pressure bottle, and this technique may be used as an alternative to boiling under a reflux. Because of the lower temperature of oxidation, a greater part of the  $\beta$ -acid is converted into products other than acetone, so that the yield of precipitate is only three-fourths as great as when the reflux is used. The results also are less constant, but it appears that when reflux apparatus is not available pressure bottles may be used if a high degree of accuracy is un-

necessary. The following experiment shows that precipitation is completed about as quickly in the pressure bottle as under the reflux.

Portions of 10 mg. of  $\beta$ -hydroxybutyric acid were placed in 400 cc. bottles with patent clamps (ordinary magnesium citrate bottles) with the reagents, including dichromate in the amounts previously prescribed for the determination of  $\beta$ -hydroxybutyric acid. The bottles were closed and nearly covered with cold water in a large kettle. The water was heated to boiling as quickly as possible. At stated intervals, measured from the moment when boiling began, bottles were removed, and the precipitates which had formed were collected and weighed in Gooch crucibles.

Time.	Precipitate.	
	Weighed.	Per gm. $\beta$ -acid.
<i>hrs.</i>	<i>gm.</i>	<i>gm.</i>
0.5	0.0372	3.72
1.0	0.0576	5.76
1.5	0.0666	6.66
2.0	0.0646	6.46
2.5	0.0664	6.64
3.0	0.0668	6.68
3.5	0.0646	6.46
4.0	0.0648	6.48

The following yields were obtained from varying amounts of  $\beta$ -hydroxybutyric acid, the time of heating at 100° being uniform at 2 hours.

$\beta$ -hydroxybutyric acid present.	Precipitate.	Precipitate per gm. $\beta$ -hydroxybutyric acid.
<i>mg.</i>	<i>mg.</i>	<i>gm.</i>
2	14.8	7.4
	14.8	7.4
10	61.8	6.18
	60.4	6.04
50	346.2	6.92
	346.6	6.93

It is evident that although good duplicate determinations with a given amount of  $\beta$ -hydroxybutyric acid are obtained, the yield of

precipitate per gm. of the acid varies much more with different amounts of the acid than when the reflux condenser is used. In case it is necessary to use the pressure bottle, 6.5 gm. of precipitate per gm. of  $\beta$ -hydroxybutyric acid may be taken as the average yield, but variations of 10 per cent in the results must be expected, unless the pressure bottle technique is worked out in more detail than it has been as presented here.

*Effect of Other Organic Substances on the Results of the  $\beta$ -Hydroxybutyric Acid Determination.*

Routine  $\beta$ -hydroxybutyric acid determinations were performed on solutions containing 10 mg. of  $\beta$ -hydroxybutyric acid with and with-

Added to 10 mg. $\beta$ -hydroxybutyric acid.			Yield of precipitate.	Effect on precipitate of added substance.	
Substance.	Amount.	Corresponding to concentration in urine sample.		Total effect.	Effect per 1 mg. added substance.
	mg.	per cent	mg.	mg.	mg.
0	0	0	84.8	—	—
0	0	0	84.8	—	—
Lactic acid.....	10.0	0.4	91.4	+6.6	+0.7
" " .....	50.0	2.0	161.6	+76.8	+1.3
Urea.....	50.0	2.0	84.6	0	0
Creatine.....	5.0	0.2	89.2	+4.4	+0.9
Uric acid.....	5.0	0.2	86.8	+2.0	+0.4
Glucose.....	20.0	0.8	80.8	-4.0	-0.2
" .....	100.0	4.0	65.8	-19.8	-0.2
"* .....	200.0	8.0	841.0	+756.2	+3.8
"** .....	500.0	20.0	233.2	+2251.4	+4.5
Thymol.....	To saturate 2.5 cc.	Saturated solution.	107.0	+22.2	—
Phenol.....	2.5	0.1	101.8	17.0	+6.8
" .....	10.0	0.4	133.2	48.4	+4.8
Ethyl alcohol....	2.5	0.1	80.6	-4.2	-1.7
" " .....	10.0	0.4	118.6	+33.8	+3.2
" " .....	100.0	4.0	1004.6	+919.8	+9.2

\* Required 0.6 gm.  $K_2Cr_2O_7$ .

\*\* Required 0.9 gm.  $K_2Cr_2O_7$ .

out the addition of other organic substances which either occur naturally in urine or might be added to it as antiseptics.

Urea in the amounts ordinarily present in urine has no effect on the yield of precipitate. Creatine (which would be converted at least partially into creatinine by the boiling in acid), lactic acid, and uric acid give precipitates, but not to such an extent that the amounts ordinarily encountered in urines would significantly affect the results obtained in ketonuria. Glucose has a peculiar effect. Amounts less than 0.1 gm. apparently interfere with the oxidative formation of acetone, as they reduce the yield of precipitate from the  $\beta$ -hydroxybutyric acid. Amounts over 0.2 gm., corresponding to 8 per cent glucose in a urine sample, yield enormous precipitates. If a solution of pure glucose is boiled with the reagents to determine acetone or hydroxybutyric acid, the solution may remain clear for 20 to 30 minutes but eventually a precipitate begins to form and increases rapidly. It is evident that glucose must be removed before the determination is performed, and we have consequently introduced its precipitation by copper into the routine technique.

The effect of alcohol is like that of glucose; small amounts decrease the yield of precipitate, larger amounts enormously increase it, doubtless from the formation of acetaldehyde. As only 2 mg. of alcohol appreciably affect the results, care must be taken that none of the flasks or pipettes used in the analysis are wet with it.

Neither thymol nor phenol (nor of course formaldehyde) may be used as preservative for urines which are to be used for these determinations.

### *Analyses of Normal Urines.*

The following analyses show the range of results for total acetone bodies that may be obtained with normal urines. The urea plus ammonia nitrogen is given as an indication of the concentration of the urine.

The maximum is 0.28 gm. per liter calculated as acetone or 0.50 gm. per liter calculated as  $\beta$ -hydroxybutyric acid. If no corrections were made for the blanks, the largest precipitate obtained, 16.8 mg., would indicate 0.42 gm. per liter calculated as acetone, or 0.75 gm.



per liter calculated as  $\beta$ -hydroxybutyric acid. While peculiar diets could doubtless cause higher figures, these may be taken as the maxima likely to be encountered in normal men under usual conditions.

Subject.	Precipitates from 25 cc. filtrate, equivalent to 2.5 cc. urine.			Total acetone bodies in urine calculated as acetone.	Urea plus ammonia nitrogen.
	Total acetone bodies precipitate uncorrected.	Blank precipitate.	Total acetone bodies precipitate minus blank precipitate.		
	mg.	mg.	mg.	gm. per liter	gm. per liter
1. V. S.....	3.0	1.4	1.6	0.04	5.77
2. G. E. C.....	1.0	2.8	1.8	0.04	9.45
3. J. A. P. ....	2.0	2.1	0.1	0.00	7.28
4. C. L.....	5.0	4.3	0.7	0.02	10.24
5. F. B.....	5.8	4.5	1.3	0.03	9.57
6. W. H.....	5.0	3.2	1.8	0.04	10.80
7. R. F.....	9.8	3.8	6.0	0.15	4.82
8. W. T.....	11.7	5.6	6.1	0.15	9.92
9. H. M.....	10.6	5.4	5.2	0.13	11.20
10. E. S.....	3.2	2.9	0.3	0.01	11.87
11. F. K.....	5.5	2.4	3.1	0.08	12.15
12. B. S.....	4.8	3.6	1.2	0.03	12.77
13. W. W. P....	11.8	8.2	3.6	0.09	—
14. A. M. L....	16.0	5.0	11.0	0.27	11.87
15. E. T.....	12.2	5.4	6.8	0.17	—
16. R. F.....	15.2	4.0	11.2	0.28	—
17. W. T.....	16.8	6.0	10.8	0.27	—
18. A. S.....	15.2	6.4	8.8	0.22	—
19. R. J. N....	10.6	9.0	1.6	0.04	—
20. D. O.....	6.8	2.6	4.2	0.10	7.61
21. W. J.....	8.8	4.4	4.4	0.11	9.07
22. S. L.....	14.0	4.2	9.8	0.24	8.81
23. H. K.....	5.0	2.0	3.0	0.07	5.32

The maximum blank determination yielded 9 mg. of precipitate equivalent to 0.22 gm. per liter of acetone, or 0.40 gm. per liter of hydroxybutyric acid.

We have also performed a considerable number of blank determinations on diabetic urine. The blanks average about the same as in normal urines (see table on p. 352).

*Analysis of Urine to Which Glucose,  $\beta$ -Hydroxybutyric Acid, and Acetone Were Added.*

3 gm. of glucose, 0.195 gm. of acetone, and 0.3182 gm. of  $\beta$ -hydroxybutyric acid (0.3952 gm. of calcium-zinc salt) were dissolved in 50 cc. of normal urine. The urine solution was poured into a 500 cc. flask, diluted with 200 cc. of water, and treated with 100 cc. of 20 per cent copper sulfate solution plus an excess of calcium hydroxide in the usual manner. 25 cc. portions of the glucose-free filtrate, equivalent to 2.5 cc. of the urine, were used for determinations performed as described at the beginning of the paper.

*Acetone plus Acetoacetic Acid Determination.*

Precipitate from urine alone.	Precipitate from urine plus added substances.	Precipitate from added substances.	Acetone calculated from precipitate.	Acetone present.
gm.	gm.	gm.	per cent	per cent
0.0014	0.1932 0.1928	0.1918 0.1914	0.383 0.383	0.390

*$\beta$ -Hydroxybutyric Acid Determination.*

Precipitate from urine alone.	Precipitate from urine plus added substances.	Precipitate from added substances.	$\beta$ -hydroxybutyric acid calculated from precipitate.	$\beta$ -hydroxybutyric acid present.
gm.	gm.	gm.	per cent	per cent
0.0028	0.1380 0.1372	0.1352 0.1344	0.640 0.638	0.6364

*Total Acetone Bodies.*

Precipitate from urine alone.	Precipitate from urine plus added substances.	Precipitate from added substances.	Precipitate calculated as acetone $\times$ 0.0200 plus $\beta$ -acid $\times$ 0.00845.
gm.	gm.	gm.	gm.
0.0040	0.3280 0.3284	0.3240 0.3244	0.3295

*Analyses of Diabetic Urines. Comparisons of Results with Those by Black's Extraction-Polarization Method.*

The data in the following table show the nature of the results obtained in conditions varying from normal to diabetic ketonuria with a nearly maximal concentration and output of acetone bodies per

TABLE.

Date.	β-hydroxybutyric acid determination.		Acetone + acetoacetic acid determination.		Total acetone bodies determination.		Total acetone bodies calculated as sum of average separate β-hydroxybutyric acid and acetone + acetoacetic determinations. Calculated as				Molecular proportion of acetone bodies in form of β-acid.	β-hydroxybutyric acid determined by Black's polarimetric method.						
	Pre-precipitate corrected.	β-hydroxybutyric acid per liter urine calculated as	Pre-precipitate corrected.	Acetone + acetoacetic acid per liter urine calculated as	Pre-precipitate corrected.	Total acetone bodies per liter urine calculated as	Total acetone bodies calculated as sum of average separate β-hydroxybutyric acid and acetone + acetoacetic determinations. Calculated as			β-acid per 24 hrs. and kg. body weight								
							gm. β-hydroxybutyric acid	cc. 0.1 M	gm. β-hydroxybutyric acid				cc. 0.1 M	gm. β-acid per liter urine	gm. β-acid per 24 hrs.			
																gm. β-hydroxybutyric acid	cc. 0.1 M	gm. β-hydroxybutyric acid
1916	liters	gm. p.p.	gm.	cc. 0.1 M	gm. β-hydroxybutyric acid	cc. 0.1 M	gm. β-hydroxybutyric acid	cc. 0.1 M	gm. β-hydroxybutyric acid	cc. 0.1 M	gm. β-acid per liter urine	gm. β-acid per 24 hrs. and kg. body weight	per cent. l. granitmet-rically deter-mined β-acid					
Feb.	20	3775	0.00360	2938	1337	13.90	7.750	0.0888	306	3.19	1.780	3828	1634	17.01	9.50	80.9	12.6	90
			0.00600	0.2970	1351	14.09	7.840	0.0888	306	3.19	1.780	0.3958	1698	17.61	9.71			
	21	3980	0.00480	0.3326	1513	15.73	8.780	0.1140	393	4.09	2.280	0.4388	1872	19.51	10.88	79.6	13.5	85
			0.00520	0.3420	1556	16.27	8.940	0.1144	394	4.11	2.290	0.4472	1910	19.91	11.00		13.7	86
	22	3620	0.00240	0.2658	1208	12.56	6.990	0.1074	370	3.85	2.150	0.3658	1561	16.27	9.07	75.9	10.9	87
			0.00280	0.2660	1210	12.57	7.000	0.1160	400	4.16	2.320	0.3752	1602	16.70	9.31			
	23	4005	0.00380	0.3436	1563	16.25	9.070	0.1235	426	4.43	2.470	0.4680	1998	20.82	11.61	79.0	15.8	96
			0.00280	0.3502	1594	16.56	9.250	0.1201	414	4.31	2.400	0.4592	1960	20.43	11.39		15.3	93
	24	3395	0.00340	0.3202	1457	15.15	8.450	0.1202	414	4.31	2.400	0.4330	1850	19.26	10.74	77.7	15.1	99
			0.00300	0.3218	1464	15.21	8.490	0.1228	424	4.41	2.460	0.4274	1825	19.03	10.60		15.3	101
	25	3375	0.00180	0.2662	1212	12.59	7.030	0.0944	326	3.39	1.890	0.3574	1525	15.90	8.85	78.9	12.6	99
			0.00280	0.2720	1238	12.86	7.180	0.0948	327	3.40	1.900	0.3546	1514	15.77	8.78		10.4	82

26	4090	0.0017	0.2318	1154	10.96	6.12	0.0691	238	2.48	1.38	0.3050	1302	13.57	7.57	14.62	59.8	1.30	83.2	
		0.0021	0.2382	1184	11.26	6.19	0.0681	235	2.44	1.36	0.3060	1306	13.61	7.59					
27	4430	0.0030	0.1843	839	8.72	4.86	0.0773	267	2.77	1.55	0.2568	1096	11.42	6.37	11.52	51.0	1.11	76.0	
		0.0012	0.1850	842	8.24	4.88	0.0769	265	2.76	1.54	0.2566	1095	11.41	6.36					
28	5610	0.0000	0.0614	279	2.91	1.62	0.0377	130	1.36	0.75	0.0994	424	4.42	2.46	4.23	23.8	0.52	68.1	2.7
		0.0000	0.0606	276	2.87	1.60	0.0378	130	1.36	0.76	0.0998	426	4.44	2.47					
29	5080	0.0004	0.0322	146	1.52	0.85	0.0155	52	0.56	0.31	0.0474	202	2.11	1.17	2.10	10.6	0.23	72.9	
		0.0004	0.0324	147	1.53	0.85	0.0170	57	0.61	0.34	0.0472	202	2.10	1.17					
Mar.																			
1	5080	0.0006	0.0216	98	1.02	0.57	0.0113	38	0.41	0.23	0.0327	140	1.45	0.81	1.41	7.2	0.16	74.3	0.8
		0.0008	0.0226	103	1.07	0.60	0.0093	32	0.33	0.19	0.0332	137	1.43	0.80					0.6
3	3450	0.0006	0.0138	63	0.65	0.36	0.0105	35	0.38	0.21	0.0268	114	1.19	0.67	1.05	3.6	0.08	64.5	0.7
		0.0004	0.0136	62	0.64	0.36	0.0099	34	0.36	0.20	0.0266	113	1.18	0.66					0.8
6	3450	0.0004	0.0028	13	0.13	0.07	0.0016	5	0.06	0.03	0.0040	17	0.18	0.10	0.20	0.7	0.01	63.2	0.4
		0.0008	0.0024	11	0.11	0.06	0.0024	8	0.09	0.05	0.0056	24	0.25	0.14					

kilo of body weight. The figures are from a patient whose alkaline reserve curves, diet, etc., are given in Chart 6 of Paper VI. All the data on this case taken together illustrate the course of an acidosis and ketonuria, both initially intense, and both successfully treated by a low calorie meat diet gradually increased to a complete fast. It may be noted that the patient's clinical condition followed the alkaline reserve rather than the ketonuria. The patient, at first with acute gastric, respiratory, and nervous signs of impending coma, became free from these symptoms as soon as the alkaline reserve approached normality, although the ketonuria continued at the same high level for several days longer. The patient was under the care of Dr. Edgar Stillman.

Blank determinations of the amounts of precipitate yielded by substances other than the acetone bodies were performed in duplicate on each urine as previously described. The results are recorded in the third column of the table, and the "corrected" weights given for the other precipitates in the case of each urine are those actually obtained diminished by the mean weight of the blank. The amounts of precipitate obtained in the blank determinations are, however, so small in proportion to the amounts obtained in the  $\beta$ -hydroxybutyric and total acetone body determinations during ketonuria that they are for practical purposes unimportant.

In the last column of the table are given for comparison the results of determinations on some of the same urines performed by Dr. Vinograd-Villchur with Black's method. The procedure as described by Black (1908) was followed without deviation except that the concentrated urines were made acid to Congo instead of to litmus, and the extraction was continued 6 hours instead of 2. It was found that unless the more acid end-point of Congo was used there was danger of only partially freeing the  $\beta$ -hydroxybutyric acid from its salts. Black's procedure is simple, and, as is seen, yielded from 85 to 100 per cent of the  $\beta$ -hydroxybutyric acid determined by our gravimetric method. That the polarimetric results are usually somewhat low is not surprising, since the known possible errors, *viz.*, incomplete extraction, the slight racemization or destruction during extraction mentioned by Shaffer and Marriott, and adsorption of

the acid by charcoal used in clearing the final solution, all are such as to lower the results.<sup>3</sup>

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<sup>3</sup> Freundlich (1906) has shown that charcoal partially adsorbs the lower fatty acids, formic, acetic, propionic, and butyric from water solution. Some adsorption of hydroxybutyric acid therefore seems probable.



## STUDIES OF ACIDOSIS.

### VIII. THE DETERMINATION OF $\beta$ -HYDROXYBUTYRIC ACID, ACETO-ACETIC ACID, AND ACETONE IN BLOOD.

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The technique for determining the acetone bodies in urine has been ascertained to be directly applicable to blood after a proper method had been found for removing the proteins from the latter.<sup>1</sup> Satisfactory results were obtained by precipitating the proteins at room temperature with the same mercuric sulfate solution (73 gm. of red mercuric oxide dissolved in 1 liter of 4 N  $\text{H}_2\text{SO}_4$ ) utilized for precipitation of the acetone. The mercury-protein precipitate leaves no interfering substances in solution, and it absorbs none of the acetone bodies: both  $\beta$ -hydroxybutyric acid and acetone added to blood are quantitatively recovered by the process described below.

*Whole Blood.*—Of whole blood 10 cc. are diluted with about 100 cc. of water in a 250 cc. flask, and 20 cc. of the 10 per cent mercuric sulfate are added. The solution is shaken for a moment, until the protein coagulates, and is then diluted with water up to the 250 cc. mark. After 15 minutes or longer it is filtered through a dry folded filter. If the first drops are cloudy they are passed through a second time. The filtrate has a slight pink tinge but the substance responsible for it does not precipitate when boiled with mercuric sulfate, nor otherwise interfere with any of the acetone body determinations.

If the blood is diluted with much more than ten volumes of water before the mercury is added, coagulation of the proteins is consider-

<sup>1</sup> Colloidal ferric hydroxide 6 cc. added cold per 1 cc. of whole blood, gives a beautiful protein-free filtrate, but the precipitate absorbs about one-third of the  $\beta$ -hydroxybutyric acid present.



ably slower,—hence the reason for not completing the dilution until after the coagulation has occurred.

*Plasma or Serum.*—8 cc. of oxalate plasma or of serum are diluted in a 200 cc. flask with 50 cc. of water and 15 cc. of the mercuric sulfate are added. The flask is shaken for a moment, until the fine precipitate which first forms has flocculated, and is then filled to the mark with water. After standing 15 minutes or longer the solution is filtered.

*Determinations.*—For determination of acetone plus acetoacetic acid or of the total acetone bodies together, 125 cc. of the filtrate, equivalent to 5 cc. of either blood or plasma, may be treated exactly as the 25 cc. of urine filtrate plus 100 cc. of water in urine analyses. The presence of mercuric sulfate in the blood filtrate interferes with the preliminary removal of acetone, so that the urine technique for  $\beta$ -hydroxybutyric acid requires modification for the blood, as described in the paragraph below.

In case however, it is desired to *determine separately the acetone plus acetoacetic acid and the hydroxybutyric acid in a single sample of blood*, this may be done by first precipitating the preformed acetone plus that from acetoacetic acid, and then determining the hydroxybutyric acid in the filtrate. The preformed acetone plus that from acetoacetic acid is precipitated exactly as in urine analysis. The filtrate from the mercury-acetone precipitate is received into a dry flask. After as much of the solution as possible has been filtered

*Factors for Calculating Results When Filtrate Equivalent to 5 Cc. of Blood Is Used for Determination.*

Determination performed.	Acetone bodies calculated as gm. of acetone per liter of blood, indicated by	
	1 gm. of precipitate.	1 cc. of 0.2 M KI solution.
Total acetone bodies.....	12.8	0.161
$\beta$ -hydroxybutyric acid.....	13.2 (14.0)*	0.172 (0.183)*
Acetone plus acetoacetic acid.....	10.0	0.130

\* These factors are used when  $\beta$ -hydroxybutyric acid is determined in the filtrate from the precipitated acetone and acetoacetic acid as described above. In this case the amount of filtrate taken from the  $\beta$ -acid determination is equivalent to only  $\frac{1}{3}$  of 5 cc. of blood, and the factor must be correspondingly increased.

through, and before any wash water has been used, 160 cc. of the filtrate, equivalent to  $\frac{160}{170} \times 5$  cc. of blood, are placed in a 500 cc. Erlenmeyer flask, heated to boiling under a reflux condenser and 5 cc. of 5 per cent potassium dichromate solution are added through the condenser. The rest of the hydroxybutyric acid determination is carried out as described for urine from the point where the dichromate is added.

To calculate the acetone bodies as  $\beta$ -hydroxybutyric acid instead of as acetone, multiply the above factors by 1.793; to calculate molecular concentration, divide the factors by 58.

Normal blood when analyzed as described for total acetone bodies yields only 1 or 2 mg. of precipitate, equivalent to 0.013 to 0.026 gm. of acetone per liter. In diabetics as much as 2.5 gm. of acetone bodies calculated as acetone has been observed while patients under ordinarily good control show 0.1 to 0.4 gm.<sup>2</sup>

<sup>2</sup> Several hundred determinations of  $\beta$ -hydroxybutyric acid and acetone plus acetoacetic acid have been performed by Dr. Fitz on the blood of diabetic patients in a study of the conditions influencing the formation of the different acetone bodies, and of the effect of the latter on the blood bicarbonate. Publication of the results is delayed by Fitz's sudden call to military duty while engaged in the problem.—D. D. V. S.



## STUDIES OF ACIDOSIS.

### IX. RELATIONSHIP BETWEEN ALKALI RETENTION AND ALKALI RESERVE IN NORMAL AND PATHOLOGICAL INDIVIDUALS.

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Sellards (1912) and Palmer and Henderson (1913) have shown that in normal individuals the administration of 5 or 10 gm. of sodium bicarbonate is sufficient to turn the urine alkaline; while in patients suffering from acidosis a greater amount is required. Palmer reports patients with uremia who received 112 gm. of bicarbonate and still excreted acid urine.

These results suggest that the kidneys secrete alkaline urine only when the bicarbonate concentration of the blood exceeds a certain level. To judge from the small amounts of bicarbonate required to turn the urine of normal men alkaline, this level would be appreciably, but not greatly above the average normal.

The work reported in the present paper was undertaken to ascertain:

1. Whether a definite level of the plasma bicarbonate does exist in normal adults, at which neutral urine is excreted, while higher bicarbonate levels cause an alkaline, and lower an acid urine. (For the sake of brevity we will term the plasma bicarbonate level at which neutral urine is excreted the *critical level*.)

2. Whether in case such a definite level exists, it is altered by disease; *i.e.*, whether in a nephritic, for example, the blood bicarbonate must be raised to a higher level than in a normal man before the urine becomes alkaline.

3. Whether absorbed or injected bicarbonate is so distributed through the body that the body weight being known, the extent to which a given dose of bicarbonate will raise the blood bicarbonate may be calculated.

That solutions of these questions have practical application as well as theoretical interest is obvious. The bicarbonate retention test of Palmer and Henderson, and Sellards is probably the simplest of all methods for the detection and approximate measurement of acidosis, and it is essential that its results should be compared with those of direct measurements of the plasma bicarbonate. The solution of (1) is necessary in order that the results of the retention test in acidosis may be intelligently interpreted in terms of internal alkali deficit; of (2) in order that the diseases, if such exist, may be known in which the retention test, because of a shift in the critical level, gives abnormal results, since in such conditions it would be unsafe to use the reaction of the urine as an indicator of the internal alkaline reserve; of (3) in order that when an alkali deficit in the blood plasma has been measured the therapeutic dose of bicarbonate required to make good the alkali deficit of the body may be estimated.

#### EXPERIMENTAL.

Sodium bicarbonate in 2 gm. amounts in 100 cc. of water was given by mouth every half hour to normal and pathological subjects until the alkalinity of the urine reached that of normal blood, a pH of approximately 7.4. In certain pathological cases, after four or five doses, if no appreciable effect on the reaction of the urine was noted the dose was increased to 5 gm. The hydrogen ion concentration of the urine was estimated before alkali was started and at half hourly intervals thereafter; and at the time of the administration of the alkali. Blood bicarbonate determinations were made just before alkali was given and again at the point when the reaction of the urine reached a pH of 7.4.

For the determination of the pH of the urine the colorimetric method described by Henderson and Palmer (1913) was employed. Plasma bicarbonate estimations were made as described by Van Slyke and Cullen.

If the sodium bicarbonate is distributed uniformly to all the body fluids we can calculate approximately the rise in blood bicarbonate caused by the absorption of 1 gm. of the salt. The calculation, in terms of plasma  $\text{CO}_2$  is made as follows: 1 gm. of  $\text{NaHCO}_3$  contains 267 cc. of  $\text{CO}_2$  measured at  $0^\circ$ , 760 mm. If the body fluids are es-

timated at 700 cc. for each kilo of body weight then the distribution of 1 gm. of bicarbonate among them would raise the  $\text{CO}_2$  content, in cc. per 100 cc. of fluid by  $\frac{267}{7W} = \frac{38}{W}$  cc.,  $W$  representing the body weight in kilos. If  $g$  gm. of bicarbonate were taken into the fluids, the rise in volume per cent of  $\text{CO}_2$  would be  $\frac{38g}{W}$ . Conversely, the amount of bicarbonate necessary to raise the  $\text{CO}_2$  by  $b$  volume per cent would be  $g = \frac{bW}{38}$ . If this equation holds even approximately, the fact shows that absorbed bicarbonate attains a fairly uniform distribution throughout the body.

We have separated our results into two groups, Table I containing the normal and Table II the pathological subjects. The several individuals are arranged in order of the number of gm. of alkali necessary to reduce the urinary reaction to that of normal blood, a pH of 7.4.

Two of the pathological cases in Table II, Experiments 17 and 19, became nauseated before the pH of the urine reached 7.4, hence the experiment was discontinued.

TABLE I.  
*Normal Subjects.*

Experiment No.	Subject.	Weight.  kg.	pH of urine.		Combined $\text{CO}_2$ in plasma.		Amount of $\text{NaHCO}_3$ given.  gm.	Calculated increase in $\text{CO}_2$ , $\frac{38g}{W}$ .  vol. per cent	Observed increase in $\text{CO}_2$ .  vol. per cent	Difference between calculated and observed $\text{CO}_2$ in- crease.  vol. per cent
			Before $\text{NaHCO}_3$ .	After $\text{NaHCO}_3$ .	Before $\text{NaHCO}_3$ .	After $\text{NaHCO}_3$ .				
					vol. per cent	vol. per cent				
1	R. T.	66	6.9	7.4	66.2	68.1	2	1.2	1.9	-0.7
2	C. L.	86	5.4	7.3	64.5	67.7	4	1.8	3.2	-1.4
3	E. S.	90	5.3	7.4	63.2	65.6	4	1.7	2.4	-0.7
4	W. W. P.	90	7.0	7.5	71.4	70.9	6	2.5	-0.5	+3.0
5	D. D. V. S.	75	7.2	7.4	68.6	74.9	6	3.0	6.3	-3.3
6	W. W. P.	90	7.2	7.4	65.5	69.8	6	2.5	4.3	-1.8
7	R. F.	70	6.2	7.5	70.7	72.8	8	4.3	1.4	+2.9
8	F. G. B.	65	5.7	7.4	69.1	75.7	10	5.9	6.6	-0.7
9	W. W. P.*	90	6.1	8.1	68.5	72.8	20	8.4	4.3	+4.1

\* The 20 gm. of  $\text{NaHCO}_3$  were taken at once. Second sample of blood 1½ hours later.

TABLE II.  
*Pathological Subjects.*

Experiment No.	Case No.	Weight. kg.	pH of urine.		Combined CO <sub>2</sub> in plasma.		Time between taking first and second blood samples.	Amount NaHCO <sub>3</sub> given.	Calculated increase in CO <sub>2</sub> , 36g. W.		Observed increase in CO <sub>2</sub> .	Difference between calculated and observed CO <sub>2</sub> increase.	Remarks.
			Before NaHCO <sub>3</sub> .	After NaHCO <sub>3</sub> .	Before NaHCO <sub>3</sub> .	After NaHCO <sub>3</sub> .			vol. per cent.	vol. per cent.			
					vol. per cent.	vol. per cent.							
10	2988	26	5.5	7.4	62.8	73.9	1½	8	11.7	11.1	+0.6	Diabetes without ketonuria.	
11	2867	64	5.1	7.4	69.5	75.8	1½	8	4.8	6.3	-1.5	Pleurisy with effusion.	
12	2793	80	7.0	7.4	76.6	77.5	3	12	5.7	0.9	+4.8	Chronic nephritis, cardiac decompensation.	
13	2907	60	5.0	7.4	68.0	77.7	3	15	9.5	9.7	-0.2	Chronic nephritis.	
14	3046	43	6.6	7.4	67.0	74.0	4	16	14.1	7.0	+7.1	Diabetes with ketonuria.	
15	2805	65	7.0	7.4	70.6	83.8	3	18	10.5	13.2	-2.7	Aortic and mitral disease. Chronic nephritis.	
16	3010	67	4.9	7.4	70.7	83.4	4	21	11.9	12.7	-0.8	Myocardial weakness; cirrhosis of liver.	
17	2961	69	5.3	7.0*	52.0	70.0	3½	30	16.5	18.0	-1.5	Chronic glomerular nephritis.	
18	2992	48	5.1	7.4	63.9	86.7	5	33	26.1	22.8	+3.3	Cardiovascular disease.	
19	2941	42	5.4	5.9*	32.5	61.4	4	37	33.5	28.9	+4.6	Diabetes with marked ketonuria.	
20	2953	37	6.9	7.3	74.7	104.4	5	38	39.0	29.7	+9.3	Diabetes without ketonuria.	

\* Became nauseated and experiment was discontinued.

#### DISCUSSION.

The results of Table I solve the first of our propositions. There is a fairly definite level of the plasma bicarbonate at which the urine changes its reaction from one more acid than blood to one more alkaline. In the normal men this occurred when the plasma bicarbonate CO<sub>2</sub> reached  $71 \pm 5$  volume per cent.

To proposition (2), concerning the critical plasma bicarbonate level in pathological cases, the solution is less satisfactory. The critical level was as a rule appreciably higher than in normal men, being in one diabetic 104.4 volume per cent. The other cases ranged

from 73.9 to 86.7. Of the nine cases in which sufficient bicarbonate was taken to raise the urinary pH to 7.4, seven showed at this point a plasma bicarbonate higher than the highest critical level shown by any of the normal men, while the other two cases (Experiments 10 and 14) were near the maximum of normal. It is evident that in disease an unusually high concentration of bicarbonate in the blood may be required to turn the urine alkaline. On the other hand, no patient showed an alkaline urine with a plasma bicarbonate below the level of normal men. The practical conclusions indicated by the facts as far as they go are that if less than 0.5 gm. of bicarbonate per kilo body weight turns the urine alkaline no acidosis exists, but *positive* retention tests for acidosis are less decisive. No. 20 (diabetic) as an extreme example, had before the test not only a normal but a high normal alkaline reserve (plasma  $\text{CO}_2 = 74.7$ ). Nevertheless because of the failure of his kidneys to secrete alkaline urine when his blood bicarbonate reached the usual critical level, he received 38 gm. or 1.03 gm. of bicarbonate per kilo, without quite raising his urine to blood alkalinity. No. 18 (cardiovascular disease) received 33 gm. or 0.79 gm. per kilo, before the urine reached blood alkalinity, although his alkaline reserve was at the average normal level (plasma  $\text{CO}_2 = 63.9$ ) at the start. In brief, it appears that when the retention test shows no acidosis, none probably exists; but when it does indicate the presence of acidosis, even our few cases show that the alarm may be false.

The behavior of the kidney to alkaline salts is quite analogous to its disturbed behavior in acid excretion (Henderson and Palmer, 1915, *a*, *b*), which leads to acid retention.

This phenomenon in connection with the use of sodium bicarbonate as a guide to the grade of acidosis in pathological cases needs further investigation. At the beginning of Experiment 15 the pH of the urine was only 7.0, while the blood plasma  $\text{CO}_2$  was 70.6 volume per cent. 18 gm. of  $\text{NaHCO}_3$ , however, were necessary to reduce the reaction of the urine to that of normal blood, to pH 7.4. This observation suggests that owing to the impaired kidney function a more acid reaction, for instance, a pH of 7.0, may be utilized in a practical way in these cases. Such utilization would of course be justified only after much more work has been done on this point.



To proposition (3) the answer is definite. The rise in plasma bicarbonate  $\text{CO}_2$  per gm. of administered bicarbonate is, except for Experiment 14, as nearly as could be expected equal to the rise calculated on the assumption that the absorbed bicarbonate is uniformly distributed to all the body fluids, the latter being calculated at 700 cc. per kilo of body weight. There are several obvious factors which make the error attending such a calculation necessarily considerable. Variability in absorption from the gastrointestinal tract into the blood stream,<sup>1</sup> distribution among the extravascular fluids, and variation in metabolism (*i.e.*, acid formation during the several hours which may be required for the test). In the normal cases, Experiments 1, 2, 3, 6, and 8 receiving 2, 4, 6, and 10 gm. of sodium bicarbonate respectively, the difference between the calculated and observed  $\text{CO}_2$  amounts to less than 2 volume per cent, which is well within the limits of the added experimental errors of the two determinations. The results obtained in Experiments 4, 7, and 9 of Table I show a discrepancy of 3 to 4 per cent between the observed and calculated effects, the difference in each case indicating that only a part of the alkali is present in the blood. Lack of complete absorption of the amount given seems the most plausible explanation in these cases.

*Calculation of Bicarbonate Dosage Necessary to Replace Observed Bicarbonate Deficits.*—From the above discussion it is quite evident that the therapeutic use of sodium bicarbonate can and should be accurately controlled. Besides causing discomfort of the patient in the form of nausea, vomiting, and even diarrhea by overdosing with bicarbonate, a severe situation may arise from producing as marked a degree of alkalosis (104.4 volume per cent), as observed in Experiment 20. Tileston (1917) reported severe tetany in a case of Weil's disease following an intravenous injection of sodium bicarbonate solution, producing a plasma  $\text{CO}_2$  of 80 volume per cent. With data at hand it is not possible to say that a high blood bicarbonate is the sole factor in producing tetany or other serious condi-

<sup>1</sup> Experiments on dogs to study the effect of intravenous sodium bicarbonate on the blood bicarbonate were started, but interrupted by the exigencies of the war.

tion, but we may reasonably interpret this finding as evidence that alkalosis very probably does play a part.

For calculating bicarbonate dosage the following table will be convenient.

Weight of individual.		Sodium bicarbonate necessary to raise plasma CO <sub>2</sub> 1 volume per cent.
kg.	lbs.	gm.
19	42	0.5
38	84	1.0
57	126	1.5
76	168	2.0
95	210	2.5

In case the individual is obese it would be logical to correct the body weight by estimating and deducting the abnormal weight.

When the organism is forming acid at a rapid rate, as in acute diabetic ketosis, the bicarbonate will raise the plasma CO<sub>2</sub> by less than the calculated amount, because part of the alkali given is neutralized by acids formed during the necessarily gradual (4 to 8 gm. per hour) administration.<sup>2</sup> Also our results (Table II) indicate that when the dosage is high (over 20 gm.) the effect may fall somewhat short of the calculated because of incomplete absorption. The figures in the table may, therefore be taken as the *minimum* doses that will produce the calculated change in plasma bicarbonate.

*Quantitative Relationship between Alkali Retention and Acidosis.*— Since the rise caused in volume per cent of plasma bicarbonate CO<sub>2</sub> by absorption of  $g$  gm. of sodium carbonate is approximately  $\frac{38g}{W}$ , and the plasma CO<sub>2</sub> at which urine normally becomes more alkaline than blood averages 71 volume per cent, the approximate relationship between plasma CO<sub>2</sub> and bicarbonate retention in the normally reacting body may be expressed by the equation: Plasma CO<sub>2</sub> = 71 —  $\frac{38g}{W}$ .

<sup>2</sup> This may have happened in Experiment 14, Table II.

Such calculations of the plasma  $\text{CO}_2$  from the retention test in pathological cases is, however, subject as discussed above to errors from abnormalities in the critical bicarbonate level of the plasma, in unusually rapid formation of acids in the body during the test, and in absorption of the orally administered bicarbonate. These errors in our extreme case (No. 20) amount to so much that they make the retention indicate the plasma  $\text{CO}_2$  as 32 volumes per cent, an acidosis of dangerous severity, when as a matter of fact no acidosis existed, the plasma  $\text{CO}_2$  being not only normal but a high normal (74.7 per cent plasma  $\text{CO}_2$ ).

However, each of the errors mentioned is such as to make the acidosis estimated from the alkali retention test greater than that actually existing. From this fact and also from the data in our tables, it appears that one may use the above formula if the plasma bicarbonate values calculated are taken only as *minimum values*. It appears, from our results and those of Palmer and Henderson, and Sellards, improbable that more severe acidosis often exists than is indicated by the bicarbonate retention test, but it may be indefinitely *less* severe than indicated. With this reservation in mind we may indicate the significance of the bicarbonate retention in terms of acidosis as follows:

Sodium bicarbonate per kilo body wt. required to turn urine alkaline.	Minimum plasma bicarbonate $\text{CO}_2$ indicated.	Maximum degree of acidosis indicated.
gm.	vol. per cent	
0.0 — 0.5	55	None.
0.5 — 0.8	55 — 40	Mild.
9.8 — 1.1	40 — 30	Moderate to severe.
Over 1.1	Below 30	Severe.

#### CONCLUSIONS.

1. In normal men the urine becomes more alkaline than the blood (pH = 7.4) when the plasma bicarbonate  $\text{CO}_2$  exceeds  $71 \pm 5$  volume per cent.

2. In most of the pathological cases studied the urine did not become more alkaline than blood until a higher plasma bicarbonate had been reached than in normal individuals. Our results show

that in pathological conditions there is danger of giving unnecessary and perhaps injurious amounts of bicarbonate if administration is continued until the urine turns alkaline. This fact may explain the disapproval under which the perfectly rational alkali therapy has fallen with some clinicians.

3. Absorbed sodium bicarbonate is distributed in approximate uniformity to the body fluids in general as well as to the blood. The effect of a given dose in raising the plasma bicarbonate may be calculated by assuming that the body contains 700 cc. of fluid per kilo and that the bicarbonate absorbed is distributed therein uniformly.

4. The results indicate the necessity of carefully controlling the therapeutic use of sodium bicarbonate. This may best be done by determination of the plasma bicarbonate. From a preliminary determination the dose necessary to restore the alkaline reserve to normality may be calculated (table on p. 367), while a subsequent determination indicates the actual effect of the administered alkali.

5. As a test for acidosis the alkali retention as used by us (bicarbonate feeding till the urine shows an alkalinity of  $\text{pH} = 7.4$ ) is subject to certain errors, all of which, especially in pathological cases, act to make the results indicate more severe acidosis than exists. The retention test indicates either the approximately correct alkaline reserve, or less. If no acidosis is indicated by the test, its absence can therefore apparently be accepted; but if acidosis is indicated, the finding must be confirmed by blood analysis before accepted.

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## STUDIES OF LUNG VOLUME. I.

### RELATION BETWEEN THORAX SIZE AND LUNG VOLUME IN NORMAL ADULTS.

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#### PLATES 3 AND 4.

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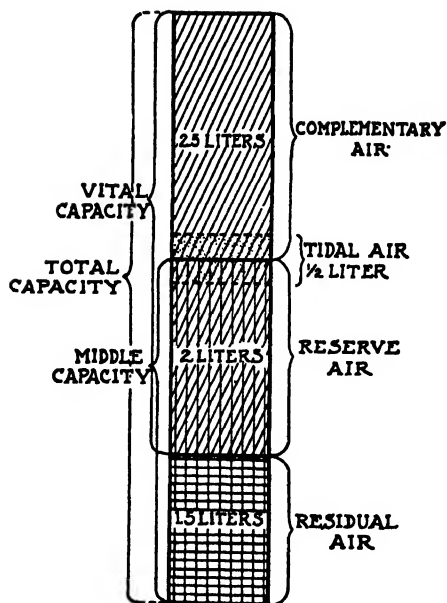
#### *Definition of Terms.*

The amount of air a person is able to expire after a maximum inspiration is called "vital capacity" (Hutchinson, 1846). The vital capacity does not, however, indicate all the air within the lungs. A certain quantity remains even after a maximum expiration; we call this "residual air" (Davy, 1800). The sum of the vital capacity and the residual air, *i.e.*, the total volume of air held by the completely filled lungs, is called the "total capacity" or "total lung volume."

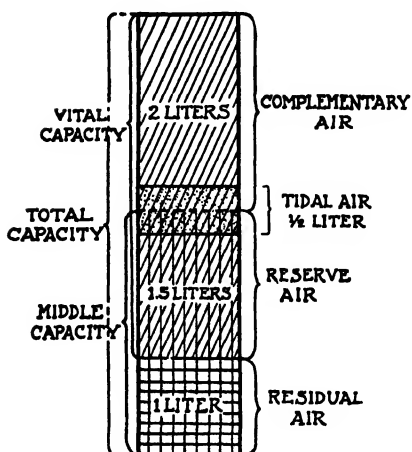
If one stops breathing half-way between a normal inspiration or a normal expiration, there will be in the lungs a certain quantity of air greater than the residual air and less than the total capacity (Text-figs. 1 and 2). We call this amount of air the "middle capacity"<sup>1</sup> (Panum, 1868). The difference between the total capacity and the middle capacity (all that can be breathed in after a half expiration) is called the "complementary air."<sup>2</sup> The difference between middle capacity and residual air (all that can be breathed out after a half expiration) is called the "reserve air."

<sup>1</sup> Siebeck suggested in 1910 defining the middle capacity as the amount of air in the lungs after a full normal expiration, instead of after a half expiration. In this paper the definition of Panum is used.

<sup>2</sup> Hutchinson (1846) created the terms "complementary air" and "reserve air." He used different definitions, however, defining the complementary air as the quantity of air a person can inspire after a normal inspiration, and the reserve air as the amount that can be expired after a normal expiration.



TEXT-FIG. 1. Approximate lung volumes for average normal man



TEXT-FIG. 2. Approximate lung volumes for average normal woman.

In accordance with the definition now in use, the vital capacity is equal to the sum of the reserve and complementary air. Under normal conditions the difference between the inspiration and expiration (the tidal air) is much less than the vital capacity, and can approximately be estimated at 500 cc. This means that a person only uses 250 cc. of his reserve air and 250 cc. of his complementary air in normal breathing. The rest of the vital capacity is to be considered as a reserve which can be used if necessary under abnormal conditions. There is a striking contrast, however, between the reserve air and the complementary air, the former always being within the chest and the latter always being outside the chest under normal resting conditions (Hutchinson).

#### *Methods for Determining Lung Volumes.*

The vital capacity, the tidal air, the reserve air, and the complementary air can be determined by means of a calibrated, easily movable spirometer. In determining the residual air, however, it is necessary to apply a more complicated method. It is usually determined by having the subject expire completely until only the residual air is left in the lungs. He then inspires from a bag or spirometer containing a known amount of nitrogen, oxygen, or hydrogen, which he mixes with the air in his lungs by respiring from five to seven times. Then the mixture is analyzed and the amount of residual air calculated from the degree to which the air in the chest has diluted the gas in the bag or spirometer. The total capacity and the middle capacity can be determined either directly by the bag alone, or indirectly by adding the residual air to the vital capacity and the reserve air respectively, as determining with a spirometer. We have, in our work, determined all the figures by means of the mixing method and later on checked the vital capacity by means of a spirometer.

Our technique has been the following: A 4 liter rubber bag is evacuated and filled with 2 liters of pure oxygen; in determining the residual air we sometimes use 3 liters. The bag is connected to a three-way stop-cock. The subject closes his lips air-tight around the rubber mouthpiece of the stop-cock. The nose is closed by a clamp. For a few respirations the stop-cock is held in such a position as to permit



free passage between the lungs and the outside air. Then the subject brings his lungs to the desired position and retains that position long enough to have the stop-cock turned to connect the rubber bag with his lungs. Four to five fairly deep respirations are sufficient to mix the air in the lungs with the air in the bag (see below). A sample is then drawn out of the bag and analyzed for nitrogen, carbon dioxide and oxygen being absorbed simultaneously by alkaline pyrogallol. The lung volume is calculated in the following way:

$$x \frac{v}{100} = (x + a) \frac{y}{100}$$

$$x = \frac{v - y}{ay}$$

$x$  = the lung volume in liters.

$v$  = the percentage of nitrogen in the lung air before the experiment (usually 79.1 per cent, see page 72).

$y$  = the percentage of nitrogen in the sample from the bag at the end of the experiment (or the percentage of nitrogen in the lungs after mixing).

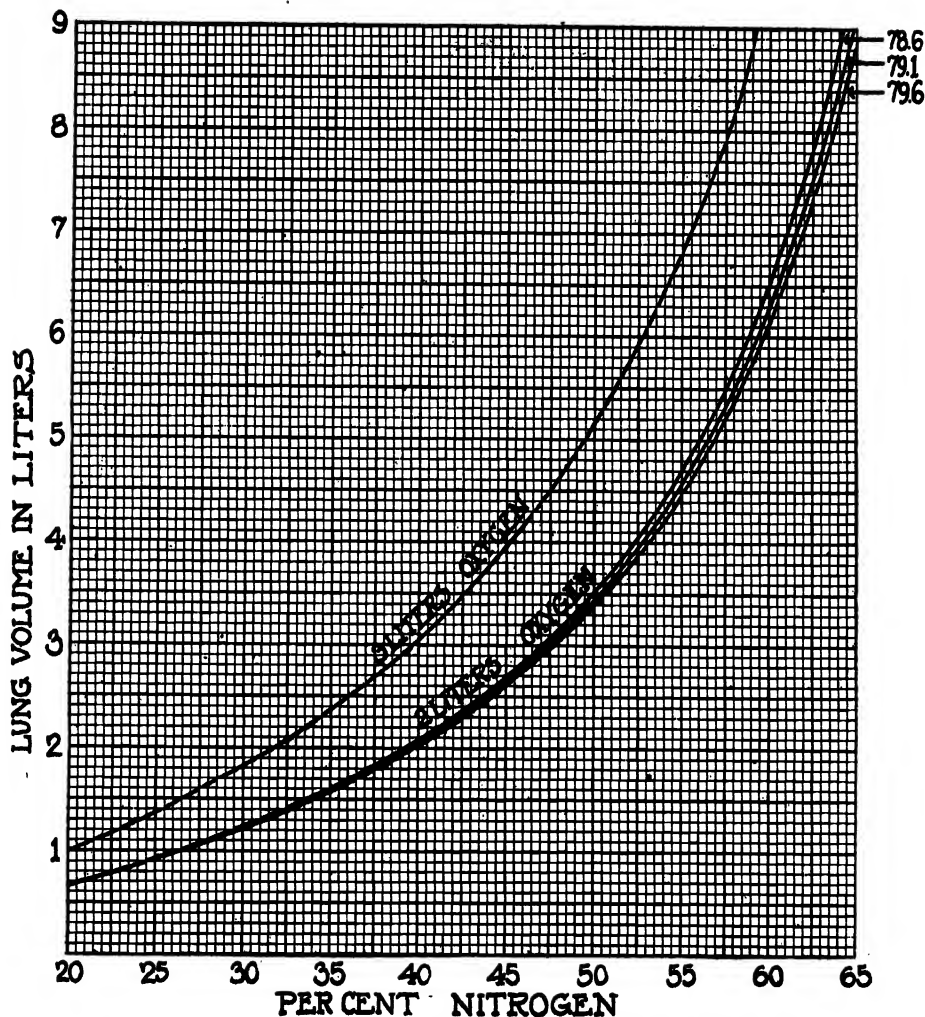
$a$  = the amount of oxygen in the bag in liters at the beginning of the experiment.

It is more convenient to calculate the lung volume by means of the curve in Text-fig. 3.

The curve and formula can only be used if the oxygen in the tank is pure or if a correction is made so that the bag will contain 2 liters of oxygen. In that case the excess nitrogen in the bag, as an impurity in the oxygen, must be subtracted from the calculated lung volume.

Our spirometer is an easily movable Krogh (1912) apparatus. The person is connected with the spirometer by means of an three-way stop-cock of at least 1 sq. cm. bore. In order to determine the vital capacity, the person must fill his lungs and stop breathing for a moment. The stop-cock is then turned and a maximum expiration is made into the spirometer. This is repeated until a constant value is obtained. The determination of the vital capacity can be combined with the determination of the residual air if a three-way stop-cock is so arranged that after expiring into the spirometer the subject is instantly connected with a rubber bag containing 2 or 3 liters of oxygen, as described above. The vital capacity can also be determined by a





TEXT-FIG. 3. Curves for calculation of lung volume (air content) as determined by the dilution method. The upper curve is for use when 3 liters of pure oxygen are mixed with the lung air, the lower when 2 liters of oxygen are used. The lower curve is given in three forms to indicate the range of error which may be caused by maximum variations in the nitrogen content of the alveolar air from the usual value of 79.1 per cent.



maximum inspiration from the spirometer, but the results obtained are somewhat smaller than those obtained by expiration (Table III).

In order to determine the reserve air and the complementary air, the person must breathe normally for some time into the spirometer, which contains 3 or 4 liters of 50 per cent oxygen to prevent dyspnea. When the breathing is regular, a maximum respiration is made. This respiration must equal the previously found vital capacity.

The figures can be given directly or in values corrected for temperature, pressure, and moisture. We have not corrected them. Our figures refer to the gas volumes measured at  $21^{\circ} \pm 3^{\circ}\text{C}$ .

### *Accuracy of Methods.*

*Spirometry.*—The spirometer method will always give the true vital capacity at that particular moment. The maximum reading error is 50 cc. Measuring the vital capacity of the same person several times, one finds that the results obtained differ by amounts usually less than 200 cc., in most cases from 3 to 6 per cent of the vital capacity. These differences are not due to the method, but to the inability of the subject to reach the same point in inspiration or expiration, or both, every time. Bohr recognized the fact that the vital capacity is not constant. He considered the maximum inspiration as a fixed point, and that the discrepancies in the determinations of the vital capacity were due to the expiration, the last part of which is done by the diaphragm. Hasselbalch showed later that it is possible to train a person to increase his total lung volume. He found, furthermore, that the total capacity and vital capacity in three normal persons decreased when they changed from the standing to the lying position. This does not exclude the fact that the maximum inspiration is a fixed point and the maximum expiration a variable point when the determinations are made within a short time and with the subject in the same position.

*Dilution Method.*—In the rubber bag method, or, as it may more accurately be called, the dilution method,<sup>3</sup> there are possibilities for several errors. The analytical error is very small because a large

<sup>3</sup> The volume of air in the lungs is determined in a way analogous to the determinations of the residue in the stomach in Ewald's test meal.

amount of air may be taken for analysis. In a determination where 30 cc. are taken for analysis, the error falls below 0.2 per cent, even when no special precautions are taken, such as the use of a thermobarometer. The main source of error is the difficulty in obtaining a homogeneous mixture of air in the lungs and in the bag. It is generally supposed that five to seven fairly deep respirations are sufficient to mix even pure hydrogen with the air in the lungs. A recent study by Sonne has shown that it is essential to pay more attention to that problem than was formerly considered necessary. Sonne found that it was very difficult, and in some instances almost impossible, to get a homogeneous mixture in the lungs by inhaling foreign air. He found it extremely difficult to get a proper mixture by mixing the lung air with nitrous oxide as is done in Krogh and Lindhard's (1912) method for determining the blood flow. Krogh and Lindhard themselves have later (1917) admitted this difficulty. We have, therefore, been very careful in controlling our results. We have done this in three different ways:

(1) By performing our experiments on the same person with different numbers of respirations (Table I). As seen in the table, increasing the number of respirations beyond four of at least 2 liter excursions does not change the results. The slight differences obtained

TABLE I.

*Effect of Variations in the Number of Respirations on Results by the Dilution Method.*

Name.	Position of chest.	Excursion of respiration.	Lung volume calculated from analyses of mixed gases after varying number of respirations.							
			1	2	3	4	5	6	7	8
		liters	liters	liters	liters	liters	liters	liters	liters	liters
Dr. F.	Maximum expiration.	2.0	1.46	1.70	2.02	1.98				
" A.	" inspiration.	3.5				1.84				5.58
" A.	Normal "	3.0				5.75				3.90
							3.85	3.96		4.00
" S.	Maximum "	3.0					6.27			3.92
" P.	" expiration.	2.0	2.16	2.45	2.49	2.50	2.50			6.20
			2.20			2.74				

are not due to an incomplete mixture, but to the previously mentioned impossibility of starting the respiration from the same point in different experiments. We have tried to overcome this difficulty in (2).

(2) By taking samples from the rubber bag after a different number of respirations in the same experiment (Table II). These experiments show that we obtained almost constant values of nitrogen in the rubber bag after four or five respirations. In all the experiments in this table the subject has started from a maximum inspiration, which probably is a more unfavorable condition for mixing than starting from a maximum expiration, because one is unable to empty

TABLE II.

*Analyses of Mixed Gases in the Bag after Varying Numbers of Successive Respirations.*

Name.	Position of chest.	Amount of pure oxygen in bag.	Excursion of respirations.	Sample 1.		Sample 2.	
				No. of respirations.	Nitro- gen.	No. of respiration.	Nitro- gen.
		<i>liters</i>	<i>liters</i>		<i>per cent</i>		<i>per cent</i>
Dr. V.	Maximum inspiration.	2 (approximately)	3½	4-6	60.5	7	60.0 60.1
" V.	" "	2	3½	4-6	59.1	8	59.4
" L.	" "	2 (approximately)	3	4-5	58.2	7	58.5
" S.	" "	2	3½	4-6	57.4	8	57.5
" S.	" "	2 (approximately)	3½	4-6	58.3	7	58.5

the rubber bag each time. The respiratory excursions in these experiments have been from 3 to 3½ liters.

(3) By determining the vital capacity as the difference between the total lung volume and the residual air, determined by the dilution method, and checking this by determining the vital capacity with the spirometer. We have done this in all but two of our subjects. The results are shown in Table III.

The values for the vital capacities determined by expiration into the spirometer are, with few exceptions, from 1 to 5 per cent greater than the values obtained by the dilution method. The reason for this is undoubtedly that we have given as our spirometer values the highest figures obtainable with the spirometer, whereas the dilution method figures are the average of all determinations. The values

for the vital capacities determined by inspiration from the spirometer are always slightly smaller than the values obtained by expiration. This is probably accounted for by the greater power of the expiratory muscles and by the resistance of the spirometer. Another reason may be the difference in temperature and moisture content of the expiratory air.

*Inconstancy of the Nitrogen Percentage in the Lung Air.*—Another possible error is due to the inconstancy of the nitrogen in the lung air and the impossibility of determining it in relation to the determination of the lung volume. We determined the nitrogen percentage in a sample from the total amount of expired air in six normal people. Six determinations were done on each person, three on the expired air after an ordinary expiration and three after a maximum inspiration. The values fell between 78.7 and 79.5 per cent in 27 cases; in 9 cases the values fell outside these limits but within 78.4 and 79.6 per cent. The variations in the same person are usually as great as from person to person. We have used 79.1 per cent in all our calculations. The curves in Text-fig. 3 show the limits of the possible errors. The constancy of the figures in Tables I and II shows that the actual errors due to increased absorption of oxygen in blood plasma and tissues from the oxygen-rich mixture breathed during the 10 to 15 seconds of the experiment, to the excretion of nitrogen from blood, and to the deviation of the respiratory quotient from 1.0 are negligible.

#### *Standard Procedure for the Determination of Lung Volumes.*

The determinations of the lung volume in Subject 2 (Table III) were done in a way which we considered the best and most reliable. It is given in detail as follows:

##### *(1) Determination of Vital Capacity by Means of Expiration in the Spirometer.—*

Expiration.	Volume. liters	Temperature. °C.	Pressure. mm.
First.....	5.75	24	762
Second .....	5.80		
Third.....	5.95		
Fourth.. . . .	5.95		
Vital capacity = 5.95 uncorrected.			



(2) *Determination of Residual Air by the Dilution Method.*—The subject breathed through a three-way stop-cock by means of which he could be connected with either the spirometer or a rubber bag containing 3 liters of oxygen. He filled his lungs with room air and breathed repeatedly into and out of the spirometer. When the volume of expiration equalled the previously determined maximum vital capacity (5.95 liters), the stop-cock was turned in such a manner that the next inspiration was made from the bag. The oxygen drawn in from the bag was rebreathed seven times in 15 seconds. The nitrogen content of the mixed gases was 32.6 per cent, indicating a residual air volume of 2.1 liters.

(3) *Total Capacity.*—The total capacity was then determined with the bag, which contained 2 liters of oxygen. The subject respired eight times. The nitrogen content of the mixture was 63.4 per cent, indicating a total capacity of 8.05 liters.

Vital capacity determined by spirometer = 5.95 liters.

Vital capacity determined by bag = 8.05 - 2.1 = 5.95 liters.

(4) *Middle Capacity.*—While breathing quietly the subject was connected with a spirometer containing about 4 liters of air with 50 per cent oxygen. He continued regular breathing from the spirometer, which registered as follows:

*Readings of the Spirometer Dial.*

	After inspiration. liters	After expiration. liters	Mean. liters	Vital capacity. liters
Normal respiration . . . .	3.2	3.9	3.55	
	3.1	3.9	3.50	
	3.15	3.85	3.50	
Maximum " . . . .	0.4	6.1	—	5.7

Reserve air = 6.1 - 3.5 = 2.6 liters.

Complementary air = 3.5 - 0.4 = 3.1 liters.

The value for the vital capacity obtained in this experiment is 6.1 - 0.4 = 5.7, instead of 5.95. The reason for this is that the maximum inspiration was made from the spirometer, a condition which, as mentioned before, regularly gives a smaller vital capacity than that registered when the lungs are filled from the free air (Table III). For this reason we increase the value of the complementary part of the total air by 0.25 to 3.35 liters.

Immediately afterwards the chest measures of the subject were taken.

*Lung Volumes in Eighteen Normal Individuals.*

The results of our experiments on eighteen normal persons between 20 and 38 years of age are tabulated in Table III, and diagrams of the

TABLE III.

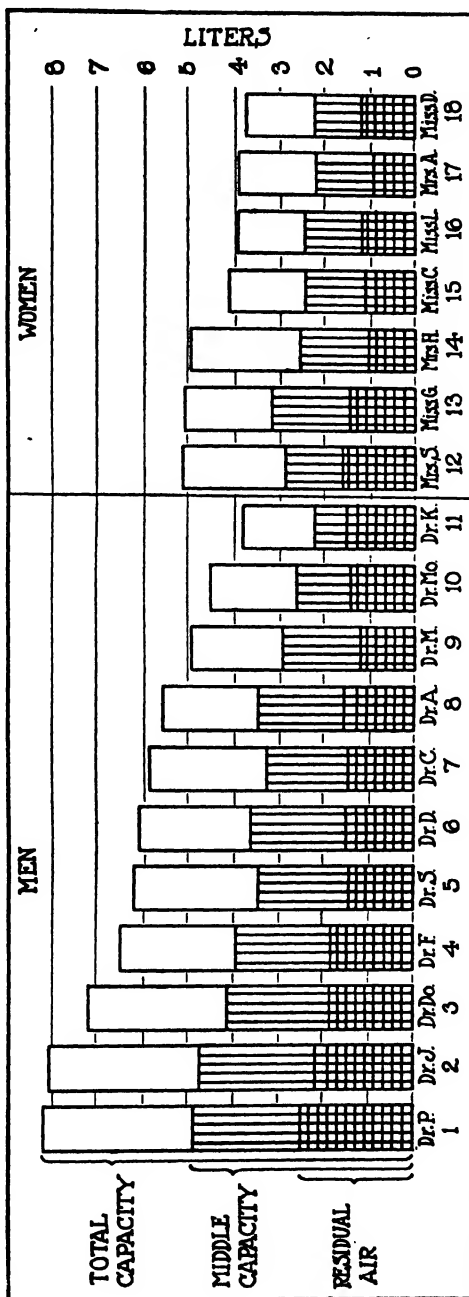
*Summary of Lung Volume Determinations on Normal Individuals.*

No. of individual.	Name.	Age. yrs.	Height. cm.	Weight. kg.	Bag.						Spirometer.	
					Residual air.	Middle capac- ity.	Total capac- ity.	Vital capac- ity.	Reserve air.	Com- plemen- tary air.	Vital capacity.	
											Expi- ration.	Inspi- ration.
					liters	liters	liters	liters	liters	liters	liters	liters
1	Dr. P.	34	185	90	2.48	4.80	8.22	5.74	2.32	3.42	5.90	5.70
2	" J.	31	179	76	2.10	4.70	8.05	5.95	2.60	3.35	5.95	5.70
3	" Do.	29	186	86	1.86	—	7.20	5.34	—	—	—	—
4	" F.	32	178	68	1.87	3.89	6.51	4.64	2.02	2.62	4.90	4.85
5	" S.	32	178	91	1.47	3.45	6.24	4.77	1.98	2.79	4.87	4.80
6	" D.	34	178	69.5	1.52	3.66	6.13	4.61	2.14	2.47	4.65	4.60
7	" C.	29	172.5	65	1.46	3.31	5.88	4.42	1.85	2.57	4.63	4.55
8	" A.	38	165	67	1.61	3.50	5.58	3.97	1.89	2.08	3.87	3.77
9	" M.	32	167.5	63	1.25	2.99	4.95	3.70	1.74	1.96	3.97	3.93
10	" Mo.	29	162.5	51	1.41	2.60	4.56	3.15	1.19	1.94	3.40	3.35
11	" K.	28	152	52	1.53	2.23	3.84	2.31	0.70	1.61	2.40	2.35
12	Mrs. S.	34	175	88	1.64	2.88	5.10	3.46	1.24	2.22	—	—
13	Miss G.	26	173	63	1.42	3.17	5.05	3.63	1.75	1.88	3.75	3.70
14	Mrs. H.	24	162	59	1.07	2.69	4.91	3.84	1.62	2.22	3.90	3.85
15	Miss C.	23	169	65	1.10	2.40	4.12	3.02	1.30	1.85	3.15	3.10
16	" L.	29	156	43	1.15	2.45	3.93	2.78	1.30	1.48	2.75	2.70
17	Mrs. A.	28	158	52	0.97	2.28	3.93	2.96	1.31	1.65	3.10	2.95
18	Miss D.	21	160	53	1.15	2.35	3.72	2.57	1.20	1.40	2.65	2.60

same determinations are shown in Text-fig. 4. The values for the different lung volumes in these determinations agree with what other investigators have found.

From his determinations on eight normal men and four normal women, Bohr derived a standard for the different lung volumes which is usually accepted.

We have divided our results into two groups according to the



TEXT-FIG. 4. Chart showing the lung volumes in eleven normal men and seven normal women.

TABLE IV.

*Average Lung Volumes for Normal Men and Women in Standing Position.*

Sex.	Residual air.	Reserve air.	Mean.	Complementary air.	Total capacity.
	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>
Men.....	1.5	2.0	3.5	2.5	6.0
Women.....	1.0	1.5	2.5	2.0	4.5

sexes and believe that the figures given in Table IV and Text-figs. 1 and 2 represent the approximate average somewhat more closely than the approximations used by Bohr; namely, 1 liter of residual, 2 liters of reserve, and 2 liters of complementary air.

*Previous Investigations to Find a Relationship between the Vital Capacity and Other Body Figures.*

Pulmometry has never played an important part in clinical medicine, chiefly on account of the great variations in the lung volumes of different persons. For that reason we have been unable to tell whether the lung volume in a pathologic case is normal or not for the individual examined unless the deviation from the usual values is great. The variations in the values of the lung volume<sup>4</sup> in different individuals have been recognized by even the earliest investigators.

Borelli (1679) was the first who tried to determine the air in the lungs. He found that from 300 to 600 cc. are taken in by a single inspiration. Jurin (1718) says about Borelli's figures: "But this quantity is different not only in different persons, but even at different times in one and the same person." Hales (1728) determined the air in the lungs to be 4 liters. Goodwyn (1788) says after reporting his own experiments: "These experiments are sufficient to show that the lungs contain a considerable quantity of air, even after complete expiration, but this quantity must vary in different subjects in proportion to the capacity of the thorax. It is, therefore, extremely difficult to establish a medium. However, we shall for the present adopt the medium quantity of these latter experiments and say that the lungs of the human subject contain 109 cubic inches (1,800 cc.)<sup>5</sup> of air after complete expiration."

<sup>4</sup> It took a considerable time before the differences between vital capacity, residual air, and so forth were recognized.

<sup>5</sup> Determined post mortem by filling the pleural cavities with water, in this way compressing the lungs (the diaphragm was fixed).

Davy (1800), who invented the dilution principle in determination of the residual air, gives the figures for his own lungs: "So that making the corrections for temperature, it would appear, that my lungs in a state of voluntary inspiration, contained about 254 cubic inches (4,160 cc.); in a state of natural inspiration, about 135 (2,210 cc.); in a state of natural expiration, about 118 (1,190 cc.); and in a state of forced expiration 41 (670 cc.)." He also remarks: "This capacity is most probably below medium; my chest is narrow, measuring in circumference but 29 inches." Hutchinson, by the invention of the spirometer, made the easily determinable vital capacity the central point in the pulmometry until the time of Bohr, half a century later. Hutchinson realized that, should the vital capacity be of any importance in clinical medicine, it was necessary to find some relationship to other body figures. For that purpose he examined 1,012 normal men and women and found that there was a certain relationship between the height and the vital capacity. He worked out the figures by means of which it should be possible to calculate the normal vital capacity from the height of the person. He showed that the weight, age, and sex might modify these figures to a slight extent. Between the vital capacity and the circumference of the chest he could not find any proportion at all. Simon (1848) confirmed Hutchinson's results as far as height was concerned, but he found that if only lean persons were used, there was some relation between the vital capacity and the circumference of the chest. Fabius (1853) found a rather close relation between the vital capacity and the volume of the trunk, which he calculated from the circumference of the chest and the distance from the neck (*eminentia occipitalis*) to the tip of the *os coccygis*. His first idea was to compare the vital capacity with the "chest volume"; he gave that up because he was unable to obtain any figures for the chest volume. Apparently without knowing Fabius' publication, Müller (1868) and Schönfeld (1882) found the same thing. The idea of Fabius (Müller and Schönfeld) did not attract much attention and was never used by others in clinical medicine. Hutchinson's old idea of calculating the vital capacity from the height prevailed and was generally used, sometimes with a slight modification of the constant and sometimes in combination with a correction for weight or age (Schneevoogt, 1854).

Wintrich (1854), Arnold (1855), von Ziemssen (1888), Cornet (1907), and Peabody (1917) have all adopted the principle laid down by Hutchinson, even if they have used it in a somewhat different way. Von Ziemssen, for instance, used a quotient (1:20 in men and 1:17 in women) to express the ratio between vital capacity and height. This quotient (Ziemssen's quotient) has been used in several papers, particularly in papers dealing with the vital capacity in people suffering from tuberculosis. Peabody divides his patients (heart patients) into four groups, according to the height.

A relationship between the residual air and other body factors has not been worked out. Only some rather rough estimates have been adopted (Schenck, 1894).

Bohr was the creator of a new era in pulmometry. He objected to the use of Hutchinson's figures for estimating the normal vital capacity from the height:

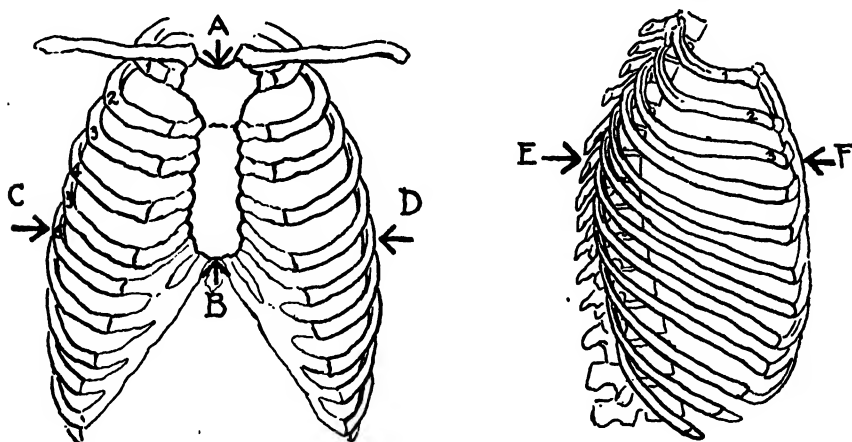
because the individual variations are too great. He furthermore objected to exclusive use of the vital capacity, because it does not take the total or residual air into consideration. He and his pupils (Hasselbalch, Rubow, Siebeck, and Bie and Maar) in several publications investigated the relation between the total lung volume, the middle capacity, and the residual air under normal and pathologic conditions, and put less stress on the absolute figures than on their relationships. The problems raised by these investigators have attracted attention for a good many years.

*Determination of the "Chest Volume" and Calculations of a Ratio between Chest Volume and Lung Volume in Different Positions of the Chest (Full Inspiration, Rest, and Full Expiration).*

The problem of finding an accurate relationship between the lung volume and chest or body size remained unsolved. It seemed to us, however, that it might be possible to approach the solution by using the chest volume as the constant and the lung volume as the variable. The reason for this seems obvious when we consider the chest wall as a sort of spirometer. When a person is respiring from a spirometer, it is a natural thing to look upon the chest wall as another spirometer connected with the first one and moving inversely to the latter. And if we take different individuals it is natural to regard their chests as spirometers containing different amounts of air.

The x-ray pictures (Figs. 1 and 2) illustrate fairly well what we mean. Fig. 1 (No. 7, Table III) is from the same person with the chest in full inspiration and expiration. Fig. 2 is from two different persons (Nos. 1 and 11) and shows the possibilities of individual variation. Our problem was to find measurements which could be used in calculating the chest volume, which alone seemed a logical basis for calculation of lung capacity. The old idea of using the chest circumference must be given up because the muscles, fat, and breasts give room for a considerable error. It seems obvious that the best way is to consider the chest as a geometrical figure and take three dimensions, the product of which will represent a volume proportional to the chest volume. We have then to measure the height, depth, and breadth of the chest and to do it in such a way that (1) the fat and muscles play as small a part as possible, (2) the different diameters represent parts of the chest wall which move in fair accordance to the respiration.

After some consideration and experiments we came to the following procedure which has been used in all our cases. The height of the chest is taken as the length of the sternum from incisio intraclavicularis to a point just below articulatio sterno-xiphoidea. The depth is then taken as the horizontal distance from the middle of the sternum at the insertion of the third rib to the spinal column, and the breadth is the distance across the sixth ribs in the midaxillary line. The points between which the measures are taken are almost without any muscular covering. The transverse points are in the axilla between



TEXT-FIG. 5. Points on the thorax where the chest measurements are taken. *AB* is the height of the chest, *CD* the breadth, and *EF* the depth.

musculus pectoralis major and musculus latissimus dorsi. The distances representing the depth and breadth vary with the phases of inspiration; the height is constant (Text-fig. 5).

The measuring requires some practice and a good deal of care. We do it in the following way: The person stands in a natural position. The points are found and marked. The point on the upper part of the sternum is rather easy to find; it is just above the edge of the bone. The lower point is sometimes very difficult to locate. We do it as follows: The curvature is found and lines are drawn to indicate it. Then we try to find the joint between the sternum and processus xiphoideus. It is usually slightly prominent. The point from which we measure is just below that prominent ridge. Locating the tip

of the processus xiphoideus sometimes helps, but it cannot always be done. The lateral points are easy to find by counting the ribs. In taking the measure it is necessary to put the nodes of the pelvimeter tight to the chest wall in order to get as close to the bone as possible. It is particularly necessary to take care that the ends of the pelvimeter do not slip and go into the intercostal spaces. The first measures are taken in rest (half-way between normal expiration and inspiration, position of middle capacity). The pelvimeter is kept on and the person is requested to take a maximum inspiration and stop a second while the measures are taken. Then he is asked to expire to the residual air and stop for another measurement. The product of the figures obtained does not, of course, represent the real chest volume, but a volume approximately proportional to it.

In Table V are given the data on different normal individuals. The product of the measurements in the three dimensions is given as the chest volume and the ratio between the chest volume and lung volume calculated as

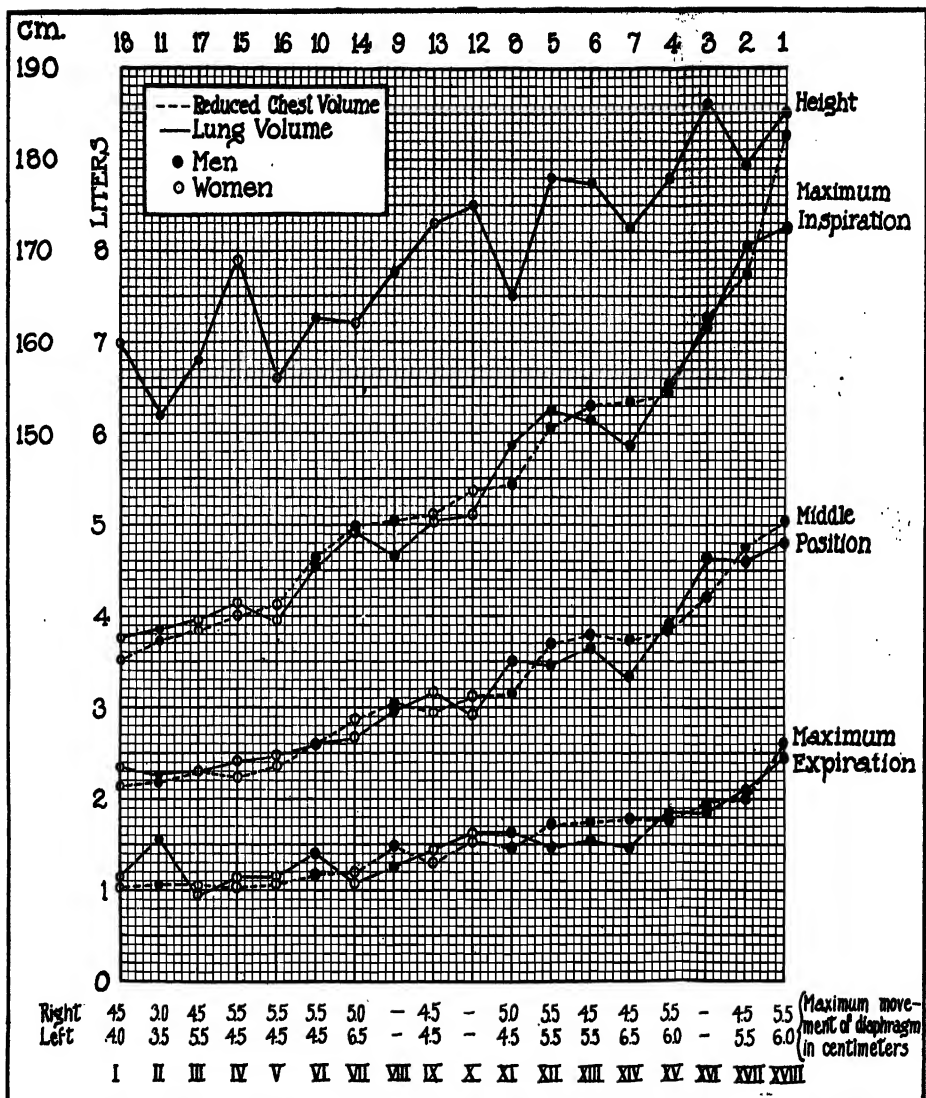
$$\frac{100 \times \text{lung volume}}{\text{chest volume}}$$

The average ratio for the total lung capacity is 55, for the middle capacity 37, and for the residual air 19.

The curves of Text-fig. 6 show the different chest volumes multiplied by these factors (reduced chest volume). It will be seen that there is a close agreement between the reduced chest volumes and the actual lung capacities. We have plotted the height on the upper part of the chart to show that the relations between the different lung volumes and the height are much more variable than the relations between the lung and chest volumes. The relationship between height and vital capacity (Hutchinson) is equally variable. The vital capacity, on the other hand, bears nearly as constant a ratio to the reduced chest volume as do the total or middle capacities. The average ratio between the vital capacity and the middle chest volume is 45 (Table VI).







TEXT-FIG. 6. Chart showing air contents of lungs (lung volumes) in normal subjects as determined in the three respiratory positions by the dilution method (solid lines) and as calculated from the thoracic measurements (broken lines). The subjects are arranged in order according to chest volumes measured at maximum inspiration. The numbers above the chart are those by which the same subjects are designated in Text-fig. 4. The numbers below indicate the maximum excursion of the right and left diaphragm in centimeters, as calculated from fluoroscopic tracings.



TABLE V.

*Summary of Thorax Measurements in the Three Positions of Respiration, Namely, Expiration, Rest, and Inspiration, and Ratios of Chest Volumes Calculated from These Measurements to Lung Volumes.*

No. of individual.	Name.	Position of chest.	Sternum.	Diameter.		Chest volume.	Lung volume.	Ratio 100 X lung volume chest volume
				Ant.-Post.	Transverse.			
			cm.	cm.	cm.	liters	liters	
1	Dr. P.	Expiration.	22.0	21.0	29.5	13.63	2.48	18.2
		Rest.	22.0	22.0	31.0	15.00	4.88	32.0
		Inspiration.	22.0	24.0	32.5	17.15	8.22	48.0
2	Dr. J.	Expiration.	21.0	17.5	29.0	10.65	2.10	19.7
		Rest.	21.0	19.5	30.5	12.49	4.70	37.6
		Inspiration.	21.0	21.0	32.0	14.08	8.05	57.2
3	Dr. Do.	Expiration.	20.5	17.0	29.5	10.28	1.86	18.1
		Rest.	20.5	18.5	30.0	11.36	4.63	—
		Inspiration.	20.5	20.0	32.0	13.12	7.20	54.8
4	Dr. F.	Expiration.	19.5	18.5	26.0	9.38	1.87	19.9
		Rest.	19.5	19.0	28.0	10.38	3.89	37.5
		Inspiration.	19.5	20.0	30.0	11.70	6.51	55.6
5	Dr. S.	Expiration.	18.0	18.5	27.5	9.15	1.47	16.1
		Rest.	18.0	19.5	28.5	10.00	3.45	34.5
		Inspiration.	18.0	20.0	30.5	11.00	6.24	56.7
6	Dr. D.	Expiration.	20.5	18.5	24.5	9.30	1.52	16.4
		Rest.	20.5	19.5	25.5	10.20	3.56	35.9
		Inspiration.	20.5	21.5	26.5	11.45	6.13	53.6
7	Dr. C.	Expiration.	19.5	17.5	27.5	9.39	1.46	15.6
		Rest.	19.5	18.5	28.0	10.10	3.31	32.6
		Inspiration.	19.5	20.0	29.5	11.50	5.88	51.1
8	Dr. A.	Expiration.	20.0	16.0	24.5	7.83	1.61	20.6
		Rest.	20.0	17.0	25.0	8.50	3.50	41.2
		Inspiration.	20.0	18.0	27.5	9.90	5.88	56.4
9	Dr. M.	Expiration.	19.5	15.5	26.0	7.86	1.25	16.4
		Rest.	19.5	16.0	26.5	8.26	2.99	36.4
		Inspiration.	19.5	17.0	27.5	9.11	4.64	51.0
10	Dr. Mo.	Expiration.	19.0	15.0	22.0	6.27	1.41	22.5
		Rest.	19.0	16.0	23.0	6.99	2.60	37.4
		Inspiration.	19.0	18.0	24.5	8.38	4.56	54.6

TABLE V—*Concluded.*

No. of individual.	Name.	Position of chest.	Sternum.	Diameter.		Chest volume.	Lung volume.	Ratio 100 × lung volume chest volume
				Ant.-Post.	Transverse.			
			cm.	cm.	cm.	liters	liters	
11	Dr. K.	Expiration.	16.0	14.5	24.0	5.57	1.53	27.5
		Rest.	16.0	15.0	24.5	5.88	2.23	37.9
		Inspiration.	16.0	17.0	25.0	6.80	3.84	56.4
12	Mrs. S.	Expiration.	17.5	18.5	24.5	7.93	1.64	20.7
		Rest.	17.5	19.0	25.2	8.38	2.88	34.3
		Inspiration.	17.5	20.2	27.5	9.72	5.10	52.4
13	Miss G.	Expiration.	18.3	14.8	25.3	6.85	1.42	20.7
		Rest.	18.3	16.5	26.1	7.88	3.17	40.3
		Inspiration.	18.3	18.5	27.3	9.29	5.05	54.2
14	Mrs. H.	Expiration.	17.2	16.0	24.8	6.83	1.07	15.7
		Rest.	17.2	17.1	25.8	7.77	2.69	34.6
		Inspiration.	17.2	18.3	28.3	8.91	4.91	55.1
15	Miss C.	Expiration.	17.9	13.9	22.2	5.52	1.10	19.9
		Rest.	17.9	14.9	22.6	6.03	2.40	39.7
		Inspiration.	17.9	15.7	25.8	7.25	4.12	55.8
16	Miss L.	Expiration.	17.1	14.9	22.5	5.73	1.15	20.0
		Rest.	17.1	16.0	23.4	6.40	2.45	38.3
		Inspiration.	17.1	17.3	25.3	7.49	3.93	52.4
17	Mrs. A.	Expiration.	16.2	14.0	23.5	5.32	0.97	18.2
		Rest.	16.2	15.5	24.5	6.15	2.28	37.1
		Inspiration.	16.2	17.1	25.4	7.04	3.93	55.4
18	Miss D.	Expiration.	17.1	14.0	22.1	5.30	1.15	21.7
		Rest.	17.1	15.0	22.2	5.79	2.35	40.3
		Inspiration.	17.1	16.5	23.2	6.55	3.72	57.8

*Excursions of the Diaphragm.*

Realizing that the measurement of the chest wall does not give us the variations in the height of the thoracic cavity, we have made a particular study of the movement of the diaphragm by means of x-rays.<sup>6</sup> The figures are given in Table VI. It will be seen that there is one man

TABLE VI.

*Maximum Excursions of the Right and Left Diaphragm.*

No. of individual.	Name.	Greatest possible movement of diaphragm.			100 X residual air chest volume at expiration	100 X vital capacity chest volume at rest
		Right.	Left.	Right + left.		
Men.						
1	Dr. P.	5.5	6.0	11.5	18.2	38.3
2	" J.	4.5	5.5	10.0	19.7	47.7
3	" Do.	—	—	—	18.1	47.0
4	" F.	5.5	6.0	11.5	19.9	43.0
5	" S.	5.5	5.5	11.0	16.1	47.7
6	" D.	4.5	5.5	10.0	16.4	45.2
7	" C.	4.5	6.5	11.0	15.6	43.7
8	" A.	5.0	4.5	9.5	20.6	46.7
9	" M.	—	—	—	16.4	44.8
10	" Mo.	5.5	4.5	10.0	22.5	45.1
11	" K.	3.0	3.5	6.5	27.5	39.2
Women.						
12	Mrs. S.	—	—	—	20.7	41.3
13	Miss G.	4.5	4.5	9.0	20.7	46.1
14	Mrs. H.	5.0	6.5	11.5	15.7	49.4
15	Miss C.	5.5	4.5	10.0	19.9	50.1
16	" L.	5.5	4.5	10.0	20.0	43.5
17	Mrs. A.	4.5	5.5	10.0	18.2	48.1
18	Miss D.	4.5	4.0	8.5	21.7	44.4

<sup>6</sup> We used fluoroscopy. Being unable to use parallel light we worked out a correction for the parallax by measuring the distance from the light to the screen (50 cm.) and the distance from the light to the middle part of the diaphragm (35 cm.). The correction is very close to 0.7 in all instances; for that reason all our directly found values have been multiplied by that factor.

with a very small movement of his diaphragm. His residual air is unusually great (Text-figs. 4 and 6). He apparently expired naturally as far as the thoracic movement was concerned, but was unable to press his diaphragm up at the end of the expiration. He was a physically untrained man, with rather undeveloped abdominal musculature. A too small movement of the diaphragm might, of course, indicate that he was unable to lower it during inspiration. The normal figures for his total capacity and the abnormally high figure for his residual air prove that this was not the case. One of the women (No. 14, Table V and Text-fig. 4) had a particularly small residual ratio, 15.7. It will be seen that the movement of her diaphragm is very extensive. She had been trained in college to breathe very deeply and had powerful abdominal muscles. She wore, like all the other women, a rather loose corset during the determination. The importance of the movements of the diaphragm will be discussed more in a later paper.

#### SUMMARY.

1. The total capacity, middle capacity, and residual air have been determined on 11 normal men and 7 normal women. All the determinations have been done on subjects in standing position and at least 2 hours after a meal.
2. The figures for the total and middle capacities agree with those of previous investigators, particularly with Bohr's. The values for the residual air seem to be a little higher than those previously published.
3. A procedure has been devised by means of which it has been possible to find a numerical relationship between external chest measurements and lung capacity.
4. With the aid of the relationship thus ascertained the lung capacity normal for a chest of given measurements can be estimated.
5. The excursions of the diaphragm have been studied.

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## EXPLANATION OF PLATES.

## PLATE 3.

FIG. 1. Two x-ray pictures from a normal man, No. 7, taken after maximum expiration (*a*) and maximum inspiration (*b*). The outline of *a* is superimposed on *b*.

## PLATE 4.

FIG. 2. Two x-ray pictures from No. 11 (*a*) and No. 1 (*b*). They are both taken in maximum inspiratory position. *a* is superimposed on *b*.



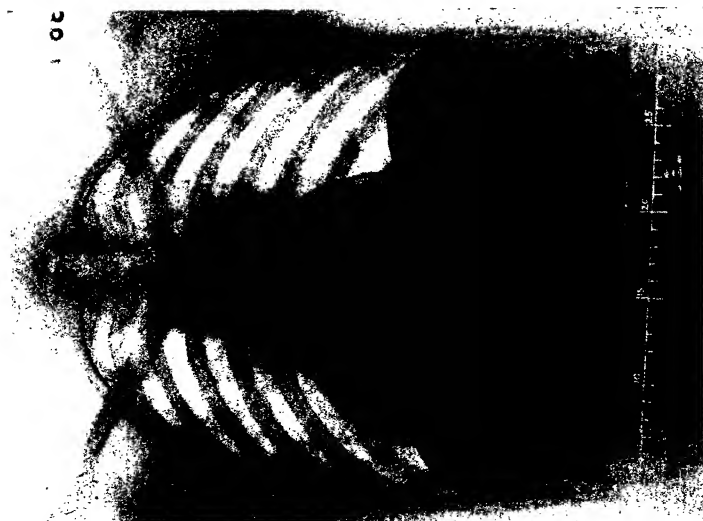


FIG. 1a.



FIG. 1b.

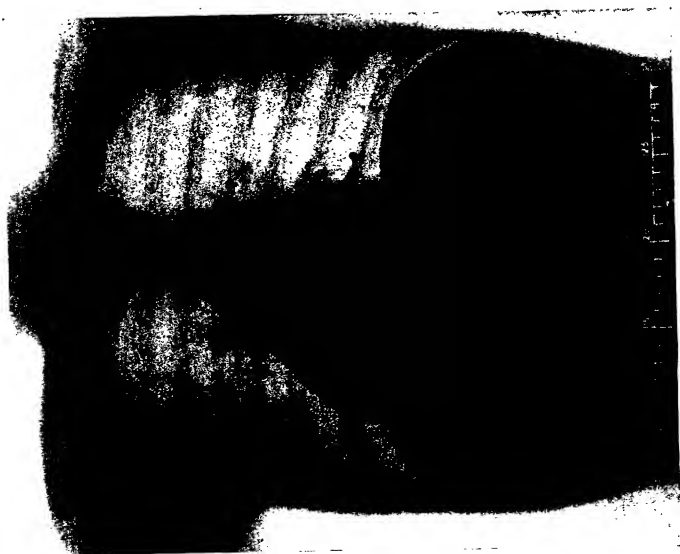


FIG. 2a.

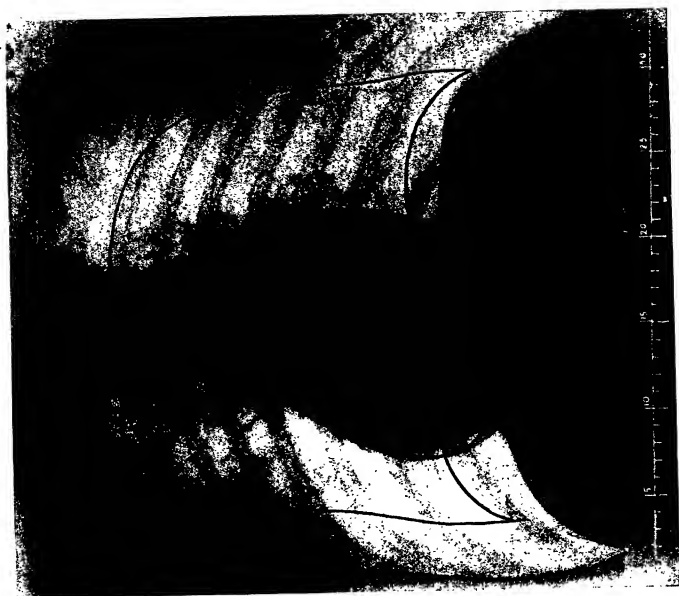


FIG. 2b.



## STUDIES OF LUNG VOLUME.

### II. TUBERCULOUS MEN.

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Hutchinson,<sup>1</sup> who invented the spirometer (1846), determined the vital capacity in twenty-two cases of early and nine cases of advanced pulmonary tuberculosis, and found that the vital capacity was subnormal in all. In the former group he found a decrease of from 10 to 50 per cent, in the latter of from 40 to 80 per cent. He calculated the normal figures from the height of the patient.<sup>2</sup> Since then numerous investigators, for example, Simon (1848), Wintrich (1854), Schneevoogt (1854),<sup>3</sup> Arnold (1855), Faivre (1864), Schönfeld (1882), and Hecht (1885), have confirmed this observation, and since von Ziemssen (1888) introduced his quotient<sup>4</sup> for the relation between height and vital capacity, spirometry has become practically a matter of routine in many clinics.

Despite the mass of data gathered, few attempts have been made to show which of the factors affecting the vital capacity are responsible for its decrease in tuberculosis.

Charlier studied the residual air in a group of patients with pulmonary tuberculosis and found it decreased. Siebeck examined the different lung volumes (total capacity, middle capacity, and residual air) in five patients and found the total capacity diminished, the middle capacity about normal, the residual air increased, and the vital capacity considerably diminished. However, the difficulty in establishing normal figures for a given pathologic case<sup>5</sup> made the de-

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<sup>1</sup> The mere title of Hutchinson's article shows how important he considered the determination of the vital capacity in patients. The title is, "On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer."

<sup>2</sup> For a detailed discussion see Lundsgaard and Van Slyke.

<sup>3</sup> Schneevoogt says: Tuberculosis of the lungs particularly will become apparent in this way before it can be diagnosed by any other means.

<sup>4</sup> Ziemssen's quotient is 1:20 in men and 1:17 in women. That means that 1 cm. of height corresponds to a vital capacity of 20 (17) cc.

<sup>5</sup> Siebeck says (p. 208): Not knowing the normal total capacity for a patient it is difficult to state anything about a deviation from the normal.

termination of absolute figures rather problematic. In other words, we do not know whether the decrease in the easily determinable vital capacity is caused by incomplete expiration (increased residual air) or by lessened inspiration (decreased total capacity). It is the purpose of the present paper partially to fill this lack in our present knowledge.

In a previous paper two of us (Lundsgaard and Van Slyke) have established evidence of a close relation between the dimensions of the chest and the capacity of the lungs in the three main positions,—maximum inspiration giving the total lung volume, rest half-way between a normal inspiration and expiration giving the middle capacity of the lungs, and maximum expiration leaving the residual air within the lungs. The ratios between the “chest volumes” and the lung volumes were worked out and found to be 55 for the total capacity, 37 for the middle capacity, and 19 for the residual air. The individual variations were within 10 per cent of these averages. Therefore, the chest volume multiplied by the factor thus determined gives the lung volume normal for a person of ascertained chest measurements.

The technique of determining the lung volumes and measuring the chest is fully described in Paper I. On the basis of our results there reported we have made an investigation of the lung volumes in 51 adult patients suffering from pulmonary tuberculosis. This paper is a report of our findings in 31 men. Our results in 20 women are reported in Paper III. The technique has been exactly as previously described. All the determinations were done with the patients in standing position. (1) The residual air was determined by the dilution method. As a rule, two determinations were done, and in some instances several. The lowest value was taken. (2) The vital capacity was then determined by expiration into a calibrated, easily movable Krogh spirometer. The expirations were continued until constancy was obtained. (3) The middle capacity was determined by normal breathing from the spirometer, which contained about 50 per cent oxygen, and the movements of the spirometer were recorded. When sufficient constancy appeared, the patient was asked to inspire and expire as much as possible, the vital capacity being controlled in this way. (4) If any doubt existed about the reliability of the experiments, a control was obtained by measuring the total capacity by the dilution method.

Having determined the lung volume, the chest measurements were taken in the three main positions, as previously described (Lundsgaard and Van Slyke). In a number the measurements were checked by two of us. Determinations of the movements of the diaphragm on a maximum respiration by means of x-ray (fluoroscopy) were then performed. The values were corrected (multiplied by 0.7) for parallax.

The other part of the investigation, the clinical examination of the patient, was completed within a few days after the lung and chest measurements. It consisted of (1) stethoscopic examination, (2) two x-ray plates, one taken from the front and one from the back, (3) determination of the influence of a certain amount of exercise on the pulse rate and respiration (Table I). The results are given on a chart for each case (Text-figs 1 to 31). The different chest measurements are reported, because the relation between them gives some information about the form of chest in each particular case. The product of these measures is called chest volume, for the sake of convenience, although, of course, it is only approximately proportional to and not equal to the real chest volume. The lung capacities in the three main positions are given, and the ratios between the chest and lung volumes calculated. Besides this the ratio between the vital capacity and the middle chest volume is calculated, the normal ratio being 35 to 47. Two columns represent in diagrammatic form the calculated lung volume<sup>6</sup> and that actually found. The lowest (cross-hatched) part indicates the residual air, the rest is the vital capacity which is divided by a line indicating the upper limit of the middle capacity. The movements of the diaphragm are given in centimeters, and two lines indicate approximately the position of the midriff in maximum expiration and maximum inspiration. The stethoscopic and roentgenological findings are shown on four diagrams of the chest wall. The following symbols are used:

*Physical Signs.*—

Light lines, slight dullness.

Heavy lines, moderate dullness.

Cross-hatching, marked dullness.

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<sup>6</sup> The calculated lung volume means simply the chest volume multiplied by the (average) ratio for normal subjects which was established in Paper I.



Fine dots, fine râles.

Larger dots, moderate and coarse râles.

Small rings, large crackling râles.

Crosses, pleuritic rubs.

Circles, antrum formation.

There is no difference in the interpretation of horizontal and vertical lines.

*X-Ray Signs.*—

Lightly shaded lines, slight density of shadow.

Heavy lines, marked density.

Circles, cavity.

Dots, stippling, the larger the dots, the coarser the stippling.

There is no difference in the interpretation of horizontal and vertical lines.

A short description of each case is also given. The observations on the pulse and respiration before and after exercise are not given in the individual charts but are collected in Table I. In order to compare the findings from the different patients, the results are presented together in Text-figs. 32, 33, and 34, in the same way as the normal individuals were shown in a previous paper.<sup>7</sup>

It will be unnecessary to discuss each patient. We have therefore divided the patients into three groups according to the severity of the objective symptoms:<sup>8</sup> the incipient, the moderately advanced, and the advanced cases.

### *Patients with Incipient Tuberculosis.*

Group I (Text-fig. 32) indicates nine patients (Nos. 1 to 9). The results in this group deviate appreciably but not greatly from the normal. The most conspicuous difference from the normal individuals is that the vital capacity<sup>9</sup> is moderately diminished in all but one case (No. 1). This is entirely due to an increase in the residual air. All the figures for the total capacity are within the normal limits; four below and five above the normal average. The residual air, on the other hand, is above the normal average in all but one

<sup>7</sup> See Text-fig. 6 of Paper I.

<sup>8</sup> We have followed the classification of The American Climatological Association which is based principally on Trudeau's scheme (cited in Rathbun, W. L., *Am. Rev. Tuberc.*, 1917, i, 13).

<sup>9</sup> See the ratio for the vital capacity on the individual charts (Text-figs. 1 to 31).

patient (No. 1). The middle capacity, which was determined in all but one patient, is not far from the normal limits. The results in this group (1) serve to confirm Hutchinson's observations<sup>10</sup> that the vital capacity was diminished even in early tuberculosis, and (2) they show that this decrease in incipient tuberculosis is not due to a diminished total lung volume, as previously supposed, but to an increased residual air. The increased residual air is the result of an inability to expire as deeply as normally. This inability to expire is apparent in the decreased movement of the diaphragm and the decreased difference between the chest volume after total expiration and in the middle position. Whether it is mechanically caused, by stiffness of the lungs, or is due to a reflex preventing compression, we cannot tell.

*Patients with Moderately Advanced and Advanced Tuberculosis.*

Group II includes thirteen moderately advanced cases (Text-fig. 33, Nos. 10 to 22) and Group III nine advanced cases (Text-fig. 34, Nos. 23 to 31). The two groups can be discussed together because the differences are not great. The picture here differs materially from that found in normal individuals and in the incipient cases. The vital capacity is diminished in all the patients, in most of them very considerably (see the value of the vital ratio in Text-figs. 1 to 31; the normal is 42). The reason for this decrease is, however, principally a decrease in the total capacity, which is only within normal limits in five patients in Group II (Nos. 11, 12, 14, 16, and 19) and two patients in Group III (Nos. 26 and 28). The residual air is, in most of the patients, fairly normal. An increase in the residual air is found only in Nos. 11, 12, 18, and 19 in Group II, and Nos. 26, 27, 28, and 30 in Group III. But, as a whole, it can be said that the vital capacity in the cases in these two groups is considerably diminished, due principally to a diminished total capacity. The cause of the diminished total capacity is not principally due to an impossibility to extend the thorax, as will be seen from the figures for the chest volumes. A comparison of the figures for the chest volumes in the three positions in the individual sub-

<sup>10</sup> Later investigators, as mentioned, have reported the same observations.

jects shows this. The essential reason is simply that the lungs do not have so much air space as in normal individuals. It is a direct expression of one phase of the pathologic anatomic process, the proliferation. Actual cavities may presumably increase the air

TABLE I.

*The Influence of Change of Position and of Exercise on Pulse and Respiration.*

No. on individual diagrams.	Case No.	Resting in bed.		Standing up.		After having run up three flights of stairs.		Other symptoms.
		Pulse.	Respirations.	Pulse.	Respirations.	Pulse.	Respirations.	
Group I.								
1	4315	72	11	106	20	110	20	None.
2	3606	66	14	70	16	98	14	Headache.
3	4362	74	16	86	20	100	18	Slight palpitation and dyspnea.
4	4280	70	14	80	18	102	28	" dyspnea; slight flush.
5	4197	96	16	106	18	120	18	" palpitation and dyspnea.
6	4184	72	15	88	18	102	20	" dyspnea.
7	4326	72	15	92	18	98	18	" "
9	4254	64	16	72	18	88	18	None.
Group II.								
10	4028	76	20	126	24	132	24	"
11	4148	102	22	98	22	108	28	Irregular pulse; slight dyspnea.
13	4229	78	24	100	22	106	20	Moderate dyspnea.
14	4090	68	14	112	14	120	14	Slight " slight palpitation.
15	4039	74	14	96	16	114	16	None.
16	3918	76	16	104	20	104	16	Moderate dyspnea; palpitation.
17	4363	80	16	100	20	110	20	Slight palpitation and dyspnea.
18	3997	88	18	100	20	120	22	Moderate dyspnea.
19	4268	64	15	102	18	102	24	Slight " "
20	4006	66	12	112	14	120	14	" " slight palpitation.
21	4076	72	18	100	18	88	20	Moderate " "
22	4082	72	8*	100	10	112	10	Slight flush.
Group III.								
25	4300	100	14	116	16	120	16	Moderate dyspnea.
26	4127	72	14	100	14	116	14	Slight " "
27	4317	98	20	110	24	120	28	Moderate " headache; flushes.
28	4346	70	14	98	16	104	14	None.
29	3952	76	12	136	14	126	16	Slight dyspnea; flush.
31	4130	112	20	120	17	120	30	Marked " tremors; flush.

\* Verified three times.

capacity of the lungs, but none of our data bears evidence of this increase. Probably the effect of cavity formation is overcome by that of the proliferation. The difference in the residual air in incipient and advanced cases is peculiar; we shall not discuss it. Previous investigators found, as we also have found, a decrease in the vital capacity corresponding to an increase in the clinical symptoms (already shown by Hutchinson in nine patients in 1846).

We attempted to discover which of the clinical signs would correspond most closely to our findings, but have given this up. However, it seems that the stethoscopic findings, particularly the extent of the râles, have a closer relation than the x-ray shadows to the decrease in total and vital capacities. More light on this problem is highly desirable. We believe that the best way to add to present knowledge will be to follow single patients over considerable periods of time, comparing the clinical findings with the pulmometry. In Table I we have given the results of our determinations of the pulse rate and respiration before and after exercise. We think that no conclusions can be drawn from them at present. It is worth mentioning that exercise influences the rate of respiration only to a small extent, whereas the pulse rate seems to be abnormally increased. The determinations of the movements of the diaphragm show a smaller excursion than we found in normal subjects.<sup>7</sup> The significance of this, as far as the lung volumes are concerned, has already been mentioned. What relation it has to the pathologic process in the lungs is not yet clear.

#### SUMMARY.

1. The total capacity, middle capacity, and residual air have been determined in 31 adult male patients suffering from tuberculosis of the lungs.

2. The chest volumes have been determined in each case and the normal lung volumes calculated by means of the ratios worked out in a previous paper.

3. In nine patients with incipient tuberculosis, the total lung volume was found within normal limits, whereas the vital capacity was diminished as a result of an increased residual air.

The increase in the residual air was due to less complete expira-

tion, caused partly by diminished movement of the diaphragm, partly by diminished compression of the chest wall. The diminished movement of the diaphragm was, as a rule, most marked on the most affected side. Whether these decreased movements are due to a reflex or to stiffness of the lung tissue we could not determine.

The middle capacity was found practically normal.

4. In twenty-two cases of moderately advanced and advanced tuberculosis, the total lung volume was in most cases markedly decreased.

The vital capacity was substantially decreased, principally as a result of the diminished total capacity. The residual air was, as a rule, normal, although in a few cases an increase in residual air also contributed to the decrease in the vital capacity.

The middle capacity, on which we do not want to put too much stress, was normal in some patients and considerably diminished in others.

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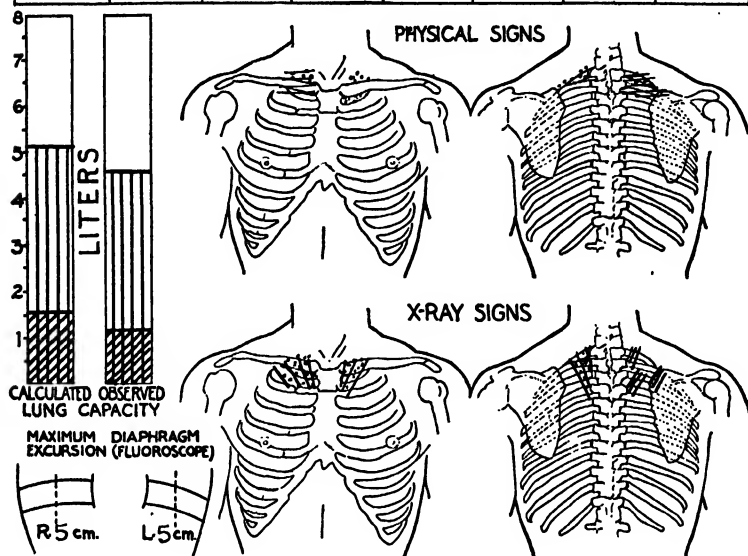
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<sup>11</sup> For the other papers quoted see the bibliography in Paper I.

# No.1 (CASE 4315)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY liters	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.8	16.1	24.9	8.30	—	—	41.0
MAX. INSP.	20.8	17.0	26.4	9.35	4.60	49.2	—
MAX. EXP.	20.8	15.8	24.7	8.13	1.20	14.8	—



TEXT-FIG. 1.

No. 1 (Case 4315).—Male, elevator operator; age 26 years. Incipient; inactive. Sputum — —, on admission, in course of treatment, and at present.

Illness began about 7 years ago with expectoration, night sweats, and pain in left chest. Moderate loss in weight; moderate dyspnea. 3 months ago slight cough, moderate expectoration, slight dyspnea, and pain at left base. His general and his lung conditions have improved satisfactorily under sanatorium treatment.

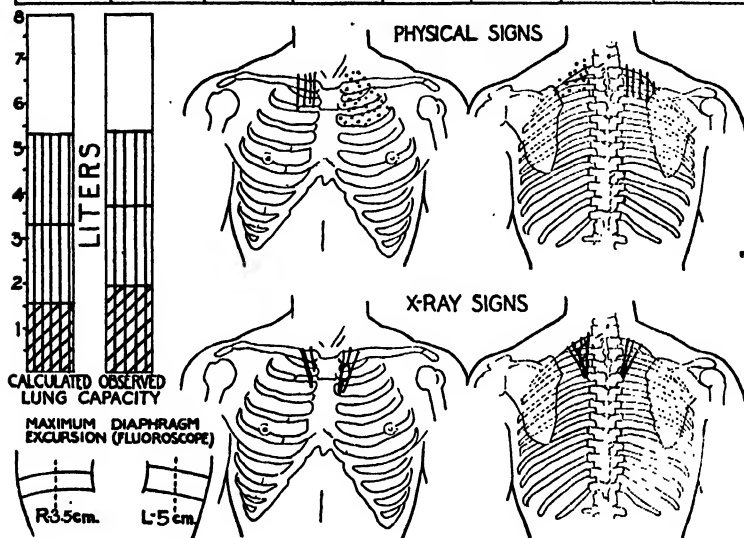
	kg.
Height 165 cm. Theoretical normal weight.....	62.0
Present weight.....	59.0
Patient's idea of normal weight.....	56.5
Date of highest weight 0 months ago.....	59.0
" " lowest " 7 " ".....	48.5
Treatment duration 3 months.	

**Physical Signs.**—April 9, 1917. Moderate dullness at right apex. No great change in breath sounds. Few coarse râles on cough above right clavicle, posteriorly a few clicks above spine of scapula. Fine râles on cough at left apex above clavicle and in the first interspace. Posteriorly a few fine râles on cough above the spine of the scapula.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace moderately infiltrated. Slight infiltration of apex and first interspace on left side. Mediastinal contents centrally placed.

## No. 2 (CASE 3606)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTEPOST.	TRANSVERSE	CHEST VOLUME* liters			
REST	cm. 20.5	cm. 18.0	cm. 23.8	liters 8.78	liters 3.67	41.7	39.8
MAX. INSP.	20.5	18.8	25.2	9.71	5.42	55.8	—
MAX. EXP.	20.5	17.1	23.3	8.17	1.92	23.4	—



TEXT-FIG. 2.

*No. 2 (Case 3606).*—Male, butcher; age 27 years. Incipient; inactive. Sputum — —, on admission, in course of treatment, and at present.

Onset 30 months ago with cough. Gastric disturbances; loss of 3 kg. in weight; marked loss in strength. Slight hemoptysis 2 years ago. Under sanatorium treatment his cough has entirely disappeared; expectoration has lessened; general physical condition improved; lung signs improved.

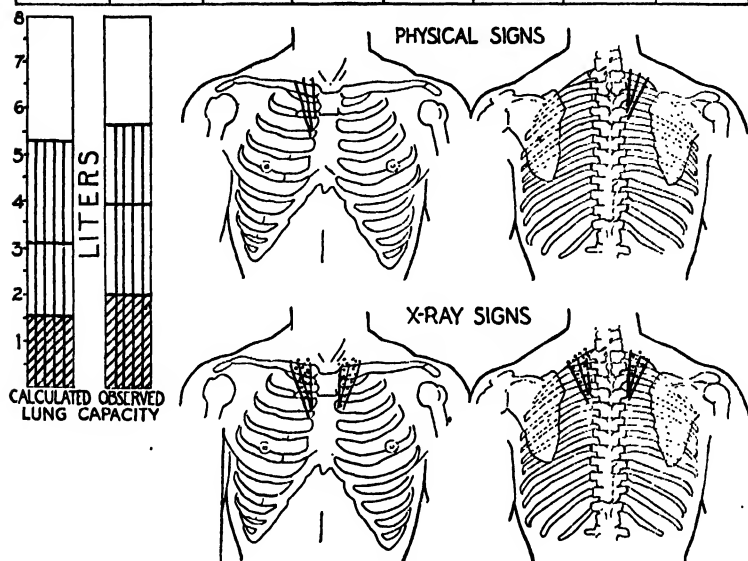
Height 174 cm.	Theoretical normal weight.....	kg. 68.0
Present weight.....		60.0
Patient's idea of normal weight.....		60.0
Date of highest weight 12 months ago.....		65.5
“ “ lowest “ 28 “ “.....		55.5
Treatment duration 22 months.		

*Physical Signs.*—April 9, 1917. Slight dullness on percussion at right apex. Breath sounds at right apex slightly harsh. No rales. No change in percussion of left lung. Breath sounds slightly weak at apex. Fine rales on cough at apex to second rib anteriorly and to the third dorsal spine posteriorly.

*X-Ray Signs.*—April 7, 1917. Right apex slightly stippled and infiltrated. Left apex densely infiltrated. Chest below inner end of clavicle has a circular cavity  $1\frac{1}{2}$  cm. in diameter. Mediastinal contents normal.

### No. 3 (CASE 4362)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL	RATIO 100 X VITAL CAP. CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	19.5	17.6	24.6	8.47	4.03	47.7	45.8
MAX. INSP	19.5	19.1	26.1	9.73	5.68	58.4	—
MAX. EXP	19.5	17.0	24.0	7.95	2.08	26.1	—



TEXT-FIG. 3.

No. 3 (Case 4362).—Male, chauffeur; age 29 years. Incipient; inactive. Sputum + on admission.

Onset 9 months ago. Malaise and tendency to tire easily; later a few night sweats loss of 2 kg. in weight. Doing well under sanatorium treatment, with no marked symptoms.

Height 173 cm.	Theoretical normal weight.....	kg. 68.5
Present weight.....	.....	62.5
Patient's idea of normal weight.....	.....	63.0
Date of highest weight 13 months ago.....	.....	63.0
“ “ lowest “ 4 “ “ .....	.....	59.5
Treatment duration 1 month.		

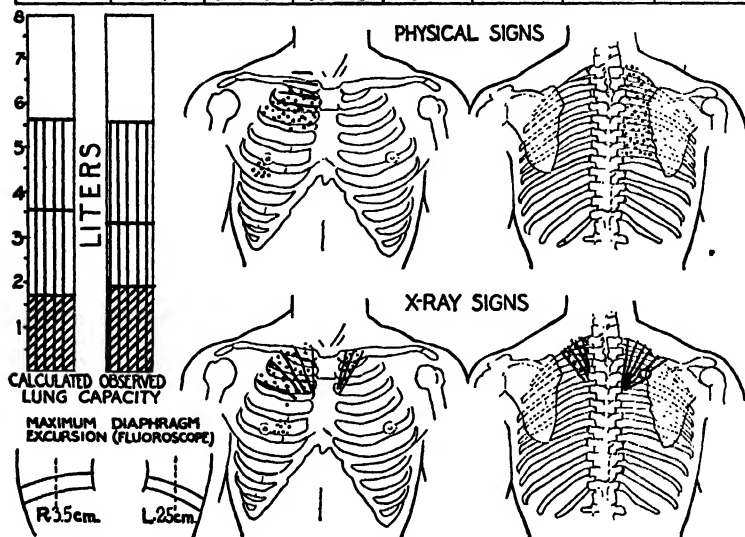
*Physical Signs.*—April 9, 1917. Slight dullness at right apex. No great change in breath sounds. No râles heard.

*X-Ray Signs.*—April 7, 1917. Right apex slight haze. Rest of lung normal. Left apex very slight haze. Rest of lung normal. Posteriorly infiltration of both apices more dense than anteriorly. Mediastinal contents normal.



# No. 4 (CASE 4280)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	19.7	18.8	26.4	9.76	3.30	33.7	37.0
MAX. INSP.	19.7	19.3	27.1	10.28	5.58	54.3	—
MAX. EXP.	19.7	18.4	25.8	9.35	2.00	21.4	—



TEXT-FIG. 4.

No. 4 (Case 4280).—Male, furrier; age 27 years. Incipient; inactive. Sputum — — —, on admission, in course of treatment, and at present.

Illness began 13 months ago with pleurisy at right base, chills, and night sweats. Cessation of symptoms after 3 weeks until 6 months ago, then return of night sweats, with considerable pain in chest. Under sanatorium treatment his general condition has remained continuously good and he has had practically no symptoms.

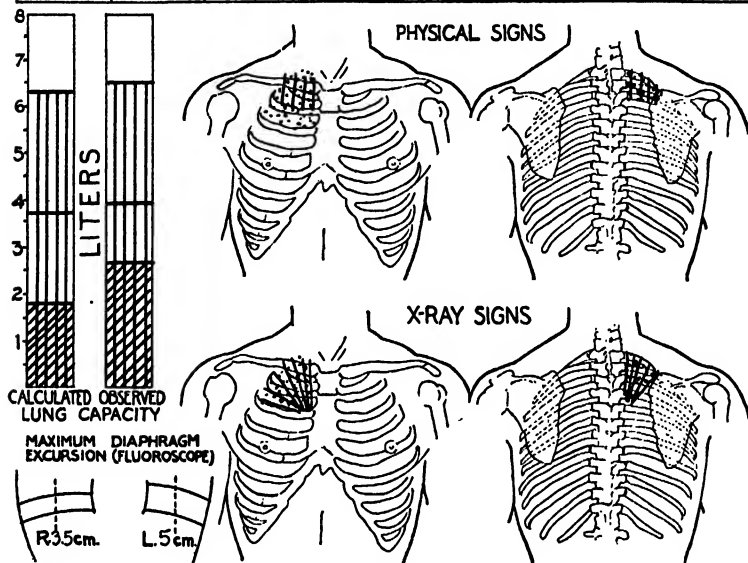
Height 171 cm.	Theoretical normal weight.....	kg. 68.0
Present weight.....		65.0
Patient's idea of normal weight.....		67.0
Date of highest weight 1913.....		68.0
“ “ lowest “ 7 months ago.....		59.5
Treatment duration 4 months.		

**Physical Signs.**—April 9, 1917. Moderate dullness at right apex to second rib. No marked change in breath sounds. Medium moist rales on cough at right apex from clavicle to third rib anteriorly, and from the apex to an inch above the angle of the scapula posteriorly. A few medium rales on cough below the right nipple.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. In the fourth interspace a small spot of stippling 3 cm. in diameter. A cavity posteriorly in the third interspace 2 1/2 cm. in diameter. Left apex slightly stippled. Mediastinal contents normal.

# No. 5 (CASE 4197)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP	RATIO 100 X VITAL CAP
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"		CHEST VOL.	CHEST VOL.
	cm.	cm.	cm.	liters	liters		
REST	21.3	18.8	25.2	10.1	3.95	39.1	38.0
MAX. INSP.	21.3	19.9	27.2	11.5	6.55	57.0	—
MAX. EXP.	21.3	18.0	25.0	9.6	2.70	28.1	—



TEXT-FIG. 5.

No. 5 (Case 4197).—Male, glove cutter; age 19 years. Incipient; inactive. Sputum — + +, on admission, in course of treatment, and at present.

Present illness began 8 months ago with moderate hemoptysis. Later moderate cough with profuse expectoration. Occasional night sweats. He has been in good general condition during his stay in the sanatorium; still has a slight cough and slight expectoration. His lung condition seems unchanged.

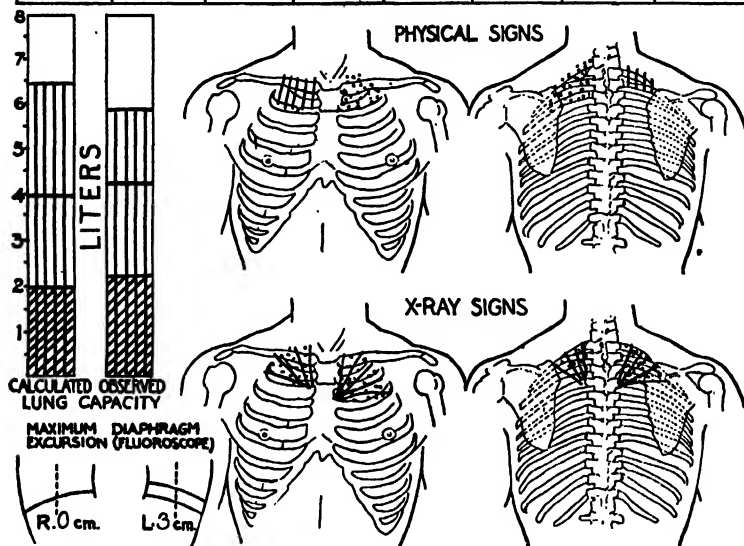
Height 175 cm.	Theoretical normal weight.....	kg. 67.5
Present weight.....		71.5
Patient's idea of normal weight.....		68.0
• Date of highest weight 4 months ago.....		72.5
“ “ lowest “ 19 “ “.....		66.0
Treatment duration 6 months.		

*Physical Signs.*—April 9, 1917. Slight dullness at right apex to second rib anteriorly and third spine posteriorly. Breath sounds slightly harsh in the same area. Fine rales on cough from the apex to the third rib anteriorly and to the third spine posteriorly.

*X-Ray Signs.*—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Mediastinal contents normal.

# No. 6 (CASE 4184)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm 22.4	cm. 16.9	cm 28.1	liters 10.7	liters 4.30	40.0	34.1
MAX. INSP.	22.4	17.9	29.1	11.7	5.85	50.0	—
MAX. EXP.	22.4	16.7	27.8	10.4	2.20	21.1	—



TEXT-FIG. 6.

No. 6 (Case 4184).—Male, machinist; age 20 years. Incipient; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Present illness began 10 months ago with malaise, slight morning cough, and expectoration; frequent night sweats. Pain in upper part of right lung. Streaked sputum occasionally for the first 3 months. His general condition has been good under sanatorium treatment; still has slight cough with moderate expectoration, occasionally blood-streaked. His lung signs have slightly increased.

Height 178 cm.	Theoretical normal weight.....	69.5
Present weight.....		75.0
Patient's idea of normal weight.....		71.5
Date of highest weight 4 months ago.....		76.0 *
" " lowest " 7 " " .....		69.0

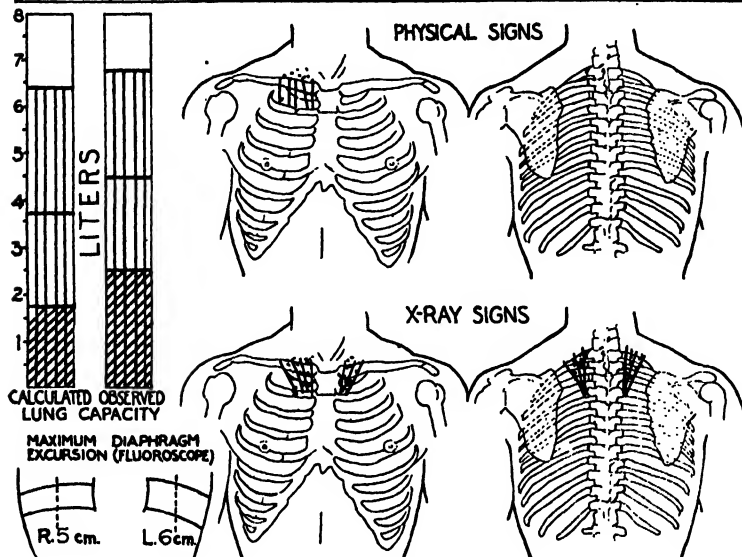
Treatment duration 6 months.

*Physical Signs.*—April 9, 1917. Slight dullness at right apex to second rib anteriorly and to the second spine posteriorly. Breath sounds slightly harsh in this area. Medium moist râles on cough at left apex to the second rib anteriorly and to the third dorsal spine posteriorly.

*X-Ray Signs.*—April 7, 1917. Right apex and first interspace slightly infiltrated. Left apex and first and second interspaces slightly stippled and infiltrated. Mediastinal contents normal.

# No. 7 (CASE 4326)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANT:POST	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.7	19.0	26.1	10.25	4.51	44.0	41.9
MAX. INSP.	20.7	20.8	27.3	11.75	6.83	58.1	—
MAX. EXP.	20.7	18.4	25.5	9.71	2.53	24.5	—



TEXT-FIG. 7.

No. 7 (Case 4326).—Male, butcher; age 38 years. Incipient; inactive. Sputum — — —, on admission, in course of treatment, and at present.

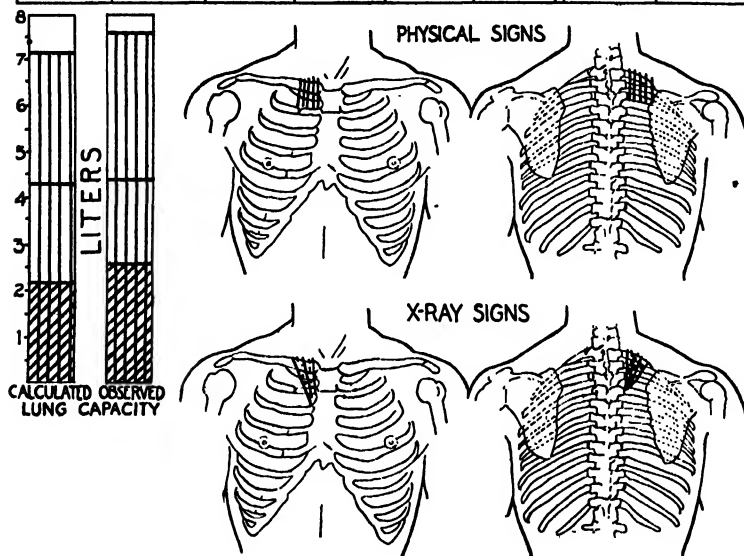
Onset 26 months ago with malaise and tendency to tire readily. 6 months ago fever, chills, slight dyspnea, slight cough, and expectoration. His general condition has been greatly improved since admission to the hospital and his lung condition markedly bettered.

Height 173 cm.	Theoretical normal weight.....	kg. 71.5
Present weight.....		68.0
Patient's idea of normal weight.....		59.0
Date of highest weight 1 month ago.....		68.5
“ “ lowest “ 4 months “.....		59.0
Treatment duration 2 months.		

*Physical Signs.*—April 9, 1917. Slight dullness at right apex, especially at inner end of first interspace. Breath sounds slightly increased at left apex, both anteriorly and posteriorly. Very few fine rales on cough at right apex above the clavicle.

*X-Ray Signs.*—April 7, 1917. Right apex moderately densely spotted and striated. Left apex moderately densely spotted and striated. Mediastinal contents normal.

No. 8 (CASE 3651)							
POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	CHEST VOLUME* liters			
REST	cm. 23.0	cm. 19.0	cm. 26.5	11.60	liters 4.37	37.7	44.0
MAX INSP	23.0	20.0	28.5	13.10	7.67	58.2	—
MAX EXP	23.0	18.0	26.0	10.60	2.57	24.2	—



TEXT-FIG. 8.

No. 8 (Case 3651).—Male, machinist; age 31 years. Incipient; inactive. Sputum + = —, on admission, in course of treatment, and at present.

Onset 6 years, 8 months ago with cough and night sweats. Loss of 4.5 kg. in weight. Frequent small hemoptyses. Expectoration slight. Under sanatorium treatment he has remained in very good general condition with good improvement in lung condition.

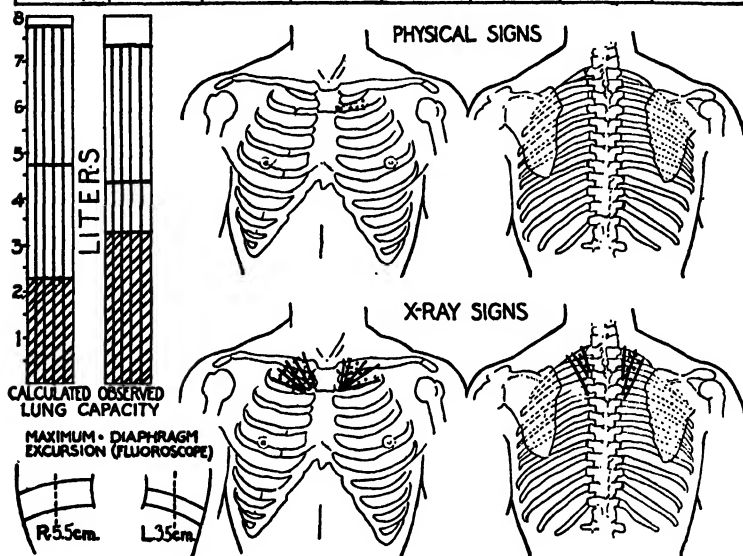
Height 183 cm.	Theoretical normal weight.....	kg. 77.0
Present weight.....		75.0
Patient's idea of normal weight.....		74.0
Date of highest weight 8 years ago.....		79.5
“ “ lowest “ 7 months “ .....		74.0
Treatment duration 22 months.		

*Physical Signs.*—April 9, 1917. Percussion resonance of right apex slightly impaired. Breath sounds slightly increased at right apex. No râles heard, before or after cough. The patient had a small hemorrhage of 5 cc. 24 hours after the measurements were taken, caused by slipping on ice.

*X-Ray Signs.*—Right apex quite densely spotted and striated. Mediastinal contents normal.

## No. 9 (CASE 4254)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY liters	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 20.0	cm. 21.1	cm. 29.8	liters 12.60	liters 4.27	33.9	32.5
MAX. INSP.	20.0	22.8	31.7	14.50	7.39	50.9	—
MAX. EXP.	20.0	20.3	29.4	11.90	3.27	27.5	—



TEXT-FIG. 9.

No. 9 (Case 4254).—Male, carpenter; age 38 years. Incipient; active. Sputum — ± —, on admission, in course of treatment, and at present.

Onset 8 months ago with cough. Expectoration slight at first, later moderate. Headaches; slight dyspnea. Loss of 3 kg. in weight. His lung condition has improved under sanatorium treatment and his general physical condition has been excellent.

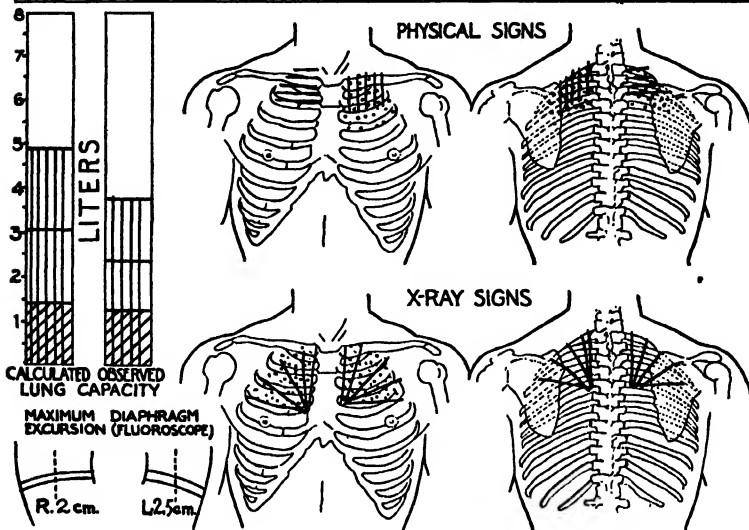
Height 170 cm.	Theoretical normal weight.....	kg. 69.5
Present weight.....		87.5
Patient's idea of normal weight.....		80.5
Date of highest weight 0 months ago.....		87.5
“ “ lowest “ 8 “ “.....		76.5
Treatment duration 5 months.		

*Physical Signs.*—April 9, 1917. No great percussion changes. No marked changes in respiratory sounds. Few fine râles on cough at the inner end of the first interspace on the left side.

*X-Ray Signs.*—April 7, 1917. Right apex and first interspace densely infiltrated. Left apex and first interspace slightly infiltrated. Mediastinal contents normal.

# No. 10 (CASE 4028)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	CHEST VOLUME*			
REST	cm. 19.0	cm. 16.9	cm. 25.5	liters 8.18	liters 2.35	28.8	29.4
MAX. INSP.	19.0	17.8	26.6	9.0	3.70	41.3	—
MAX. EXP.	19.0	15.7	25.3	7.56	1.30	17.2	—



TEXT-FIG. 10.

No. 10 (Case 4028).—Male, student; age 17 years. Moderately advanced; active. Sputum — + +, on admission, in course of treatment, and at present.

Onset 13 months ago with a cold. Cough severe; expectoration slight. Moderate dyspnea. Occasional chills. 2 weeks after onset a profuse hemoptysis. Loss of 2.7 kg. in weight. His symptoms remain about the same under sanatorium treatment. Physical signs have increased. General condition remains fair.

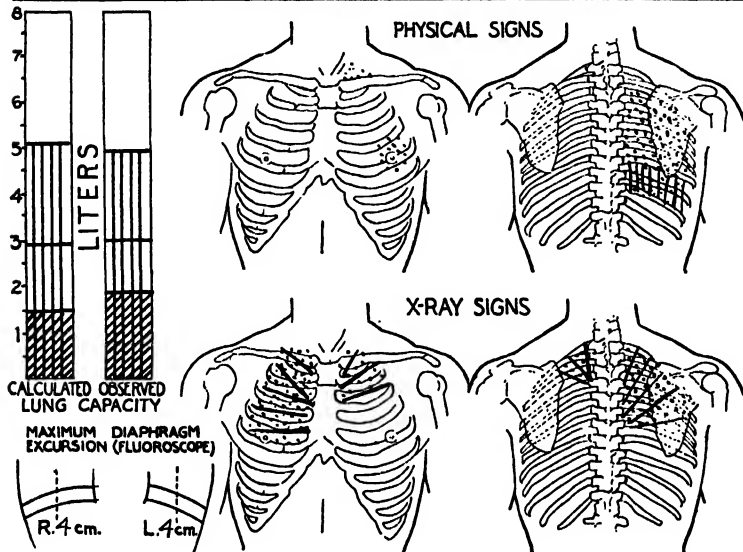
Height 168 cm.	Theoretical normal weight.....	57.5
Present weight.....		61.0
Patient's idea of normal weight.....		59.0
Date of highest weight 0 months ago.....		61.0
“ “ lowest “ 7 “ “ .....		48.0
Treatment duration 11 months.		

**Physical Signs.**—April 9, 1917. Moderate dullness at right apex to second rib anteriorly and third spine posteriorly. Slight dullness at left apex to second rib anteriorly and third spine posteriorly. Breath sounds moderately harsh at right upper thorax. Breath sounds feeble at left upper thorax. Râles on cough, fine and medium, at right apex to fourth spine posteriorly. Medium râles on cough at left apex to third rib anteriorly and fourth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first, second, and third interspaces moderately stippled. Left apex and first, second, and third interspaces moderately stippled. Mediastinal contents normal.

# No. 11 (CASE 4148)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	19.2	17.2	24.1	7.92	3.05	38.5	37.7
MAX. INSP	19.2	18.6	25.9	9.25	4.90	53.0	—
MAX. EXP.	19.2	16.8	23.9	7.72	1.90	24.6	—



TEXT-FIG. 11.

No. 11 (Case 4148).—Male, freight house clerk; age 23 years. Moderately advanced; inactive. Sputum + = —, on admission, in course of treatment, and at present.

Onset 18 months ago with hemoptysis. Cough moderate; expectoration slight. Few night sweats. Loss of 4.5 kg. in weight. During his sanatorium stay he has remained in good general condition. Lung condition slightly improved.

Height 175 cm.	Theoretical normal weight.....	kg. 69.0
Present weight.....		59.5
Patient's idea of normal weight.....		56.0
Date of highest weight 1 month ago.....		60.5
“ “ lowest “ 7 months “ .....		50.5
Treatment duration 7 months.		

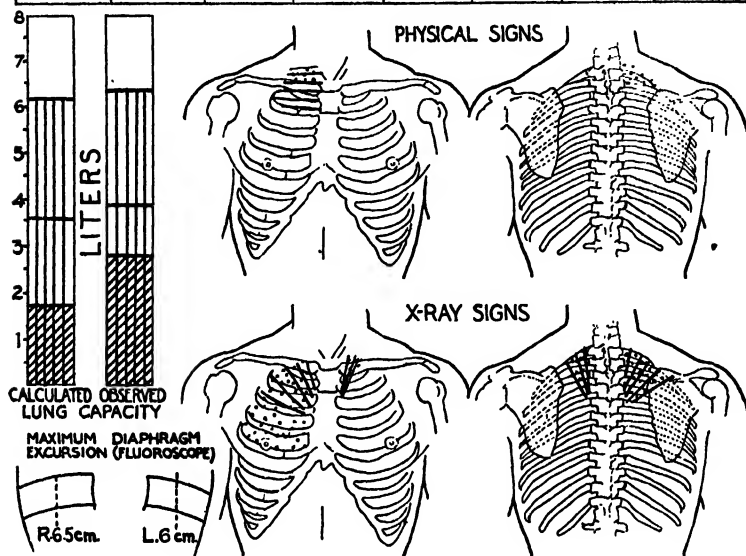
**Physical Signs.**—April 9, 1917. Slight dullness at right base posteriorly. No great change in breath sounds. Fine moist râles on cough at left apex above the clavicle. Fine moist râles on cough at the left anterior third and fourth interspaces in the region of the nipple. Fine moist râles on cough posteriorly on the right side from the spine of the scapula above to the base.

**X-Ray Signs.**—April 7, 1917. Right, fine stipplings from the apex to the fourth interspace. Left, moderate stippling from the apex to the second interspace. Mediastinal contents slightly to the right.



# No.12 (CASE 3990)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP	RATIO 100 X VITAL CAP
	STERNUM	ANTE-POST.	TRANSVERSE	*CHEST VOLUME*		CHEST VOL.	CHEST VOL.
REST	cm. 20.0	cm. 20.0	cm. 24.0	liters 9.60	liters 3.89	40.5	37.5
MAX. INSP.	20.0	22.0	25.5	11.20	6.34	56.7	—
MAX. EXP.	20.0	19.5	23.5	9.20	2.74	29.8	—



TEXT-FIG. 12.

No. 12 (Case 3990).—Male, machinist; age 25 years. Moderately advanced; inactive Sputum — — —, on admission, in course of treatment, and at present.

Onset 30 months ago with hemoptysis. Loss of 4 kg. in weight. Pain in chest slight. Slight dyspnea. Slight expectoration. During his hospital stay he has remained in fair general condition with moderate improvement in lung condition.

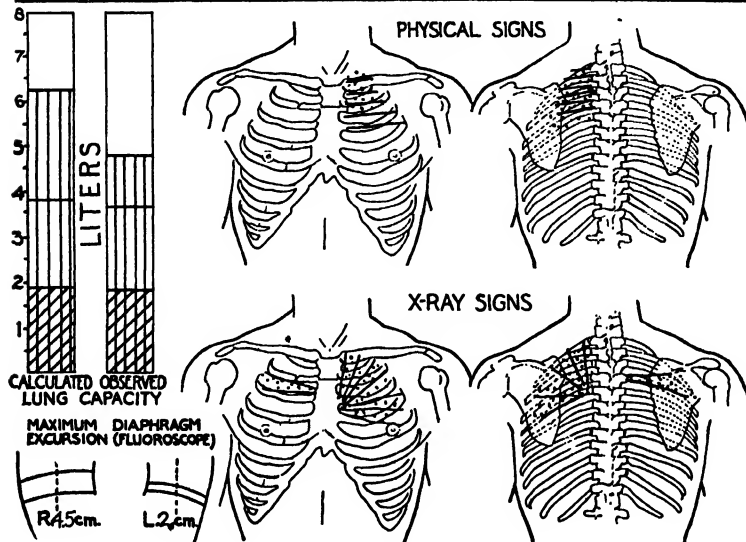
Height 170 cm.	Theoretical normal weight.....	kg. 65.5
Present weight.....		65.5
Patient's idea of normal weight.....		60.5
Date of highest weight 10 months ago.....		68.0
“ “ lowest “ 2 years “ .....		58.5
Treatment duration 12 months.		

**Physical Signs.**—April 9, 1917. Dullness at upper part of right lung anteriorly to the second rib. Increased breath sounds at upper part of right lung anteriorly to the second rib and posteriorly to the spine of the scapula. Medium moist râles on cough at the right apex, above the clavicle anteriorly, and to the spine of the scapula posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces quite densely spotted and striated. The third and fourth interspaces show very fine spottings. Left apex slightly spotted; rest of lung normal. Mediastinal contents normal.

# No.13 (CASE 4229)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.0	18.8	26.1	10.30	3.75	36.4	28.1
MAX. INSP.	21.0	19.7	27.4	11.35	4.70	41.4	—
MAX. EXP.	21.0	18.4	25.8	10.00	1.80	18.0	—



TEXT-FIG. 13.

No. 13 (Case 4229).—Male, music teacher; age 42 years. Moderately advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 12 months ago with heavy cold. Cough moderate; expectoration profuse after 2 weeks. Dyspnea moderate. Loss of 2.7 kg. in weight. 2 months after onset profuse hemoptysis. Pain in left side. Under treatment in the hospital he has improved in general condition; cough and expectoration moderate. The physical signs remain about the same.

	kg.
Height 170 cm. Theoretical normal weight.....	70.0
Present weight.....	65.0
Patient's idea of normal weight.....	61.0
Date of highest weight 2 months ago.....	65.5
" " lowest " 5 " " .....	58.5

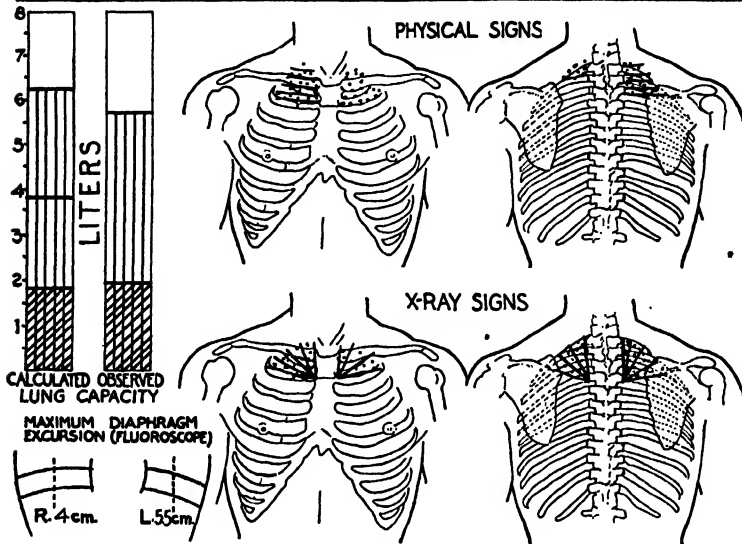
Treatment duration 5 months.

**Physical Signs.**—April 9, 1917. Moderate dullness on percussion over the left apex and from the apex to the third rib anteriorly and to the fourth spine posteriorly. Breath sounds moderately harsh in this area. Rales on coughing, fine and medium, from apex to the second rib anteriorly and to the fifth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right lung, second interspace slightly stippled. Left upper lobe moderately densely spotted and stippled. Mediastinal contents slightly to the left above.

# No. 14 (CASE 4090)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	20.5	18.7	27.0	10.4	—	—	32.7
MAX. INSP.	20.5	20.0	27.9	11.4	5.7	50.0	—
MAX. EXP.	20.5	17.8	26.2	9.6	2.0	20.9	—



TEXT-FIG. 14.

No. 14 (Case 4090).—Male, lithographer; age 32 years. Moderately advanced; inactive. Sputum + = +, on admission, in course of treatment, and at present.

Present illness began 13 months ago with neurasthenic symptoms, insomnia, etc. 2 months later slight cough with scanty expectoration; no other symptoms. Under sanatorium treatment his cough has lessened. Has complained of insomnia. General physical condition has bettered. His lung signs have improved slightly.

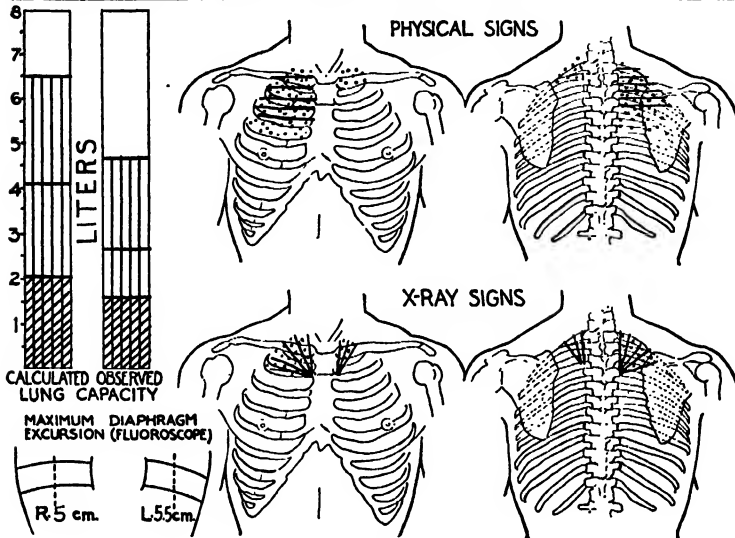
Height 177 cm.	Theoretical normal weight.....	kg. 74.5
Present weight.....		76.0
Patient's idea of normal weight.....		72.5
Date of highest weight 8 years ago.....		85.0
“ “ lowest “ 7 months “ .....		69.5
Treatment duration 9 months.		

**Physical Signs.**—April 9, 1917. Moderate dullness at right apex to second rib anteriorly and to third spine posteriorly. Moderately harsh breathing in this area. Breath sounds slightly feeble at left apex. Fine râles at right apex to second rib anteriorly and to third spine posteriorly. Fine râles on cough at left apex to second rib anteriorly and to second spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Left apex and first interspace similarly infiltrated. Mediastinal contents normal.

# No. 15 (CASE 4039)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 21.5	cm. 19.6	cm. 26.5	liters 11.2	liters 2.65	23.7	26.9
MAX. INSP	21.5	20.0	27.4	11.8	4.6	39.0	—
MAX. EXP.	21.5	18.9	26.1	10.6	1.6	15.0	—



TEXT-FIG. 15.

No. 15 (Case 4039).—Male, clothing cutter; age 28 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 6 years ago with cold. Persistent cough. Hemoptysis slight 6 months after onset. 2 years ago series of severe hemoptyses. Artificial pneumothorax. Has felt well and is near his normal weight since recovery after hemoptyses. Under sanatorium treatment his symptoms have remained slight; very little cough, slight expectoration. His physical condition has been excellent.

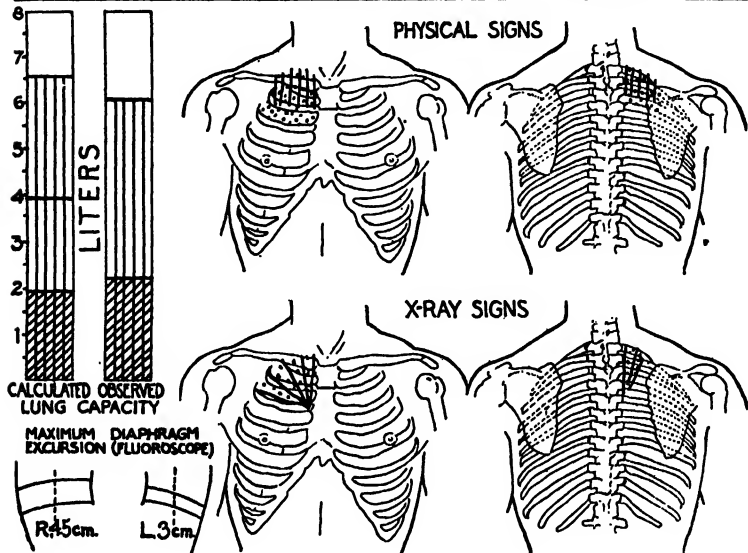
Height 175 cm.	Theoretical normal weight.....	kg. 70.5
Present weight.....		72.0
Patient's idea of normal weight.....		72.5
Date of highest weight 4 years ago .....		77.0
" " lowest " 28 months " .....		54.5
Treatment duration 10 months.		

**Physical Signs.**—April 9, 1917. Moderate dullness on percussion at right apex to the third rib anteriorly and to the fourth spine posteriorly. Breath sounds moderately harsh. Râles on cough, fine and medium, at apex to the fourth rib anteriorly and to the sixth spine posteriorly. Breath sounds slightly feeble at left apex. Medium moist râles on cough at apex to second rib anteriorly and to the second spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace densely infiltrated. Left apex slightly infiltrated. Mediastinal contents normal.

# No.16(CASE 3918)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE.POST.	TRANSVERSE	"CHEST VOLUME" liters			
REST	cm. 20.4	cm. 18.1	cm. 29.4	10.75	—	—	34.8
MAX. INSP.	20.4	19.1	30.8	11.93	6.10	51.3	—
MAX. EXP.	20.4	17.6	28.9	10.38	2.25	21.6	—



TEXT-FIG. 16.

No. 16 (Case 3918).—Male, factory inspector; age 27 years. Moderately advanced; inactive. Sputum + + —, on admission, in course of treatment, and at present.

Onset 19 months ago with malaise, loss in strength; 2 months later fever and cough. Sputum occasionally blood-streaked. Loss of 3 kg. in weight. Occasional night sweats. Dyspnea slight. Under sanatorium treatment he has remained in fair general condition. Complications of larynx and rectal fistula have arisen. Slight hemoptysis frequent. Symptoms and lung condition remain the same.

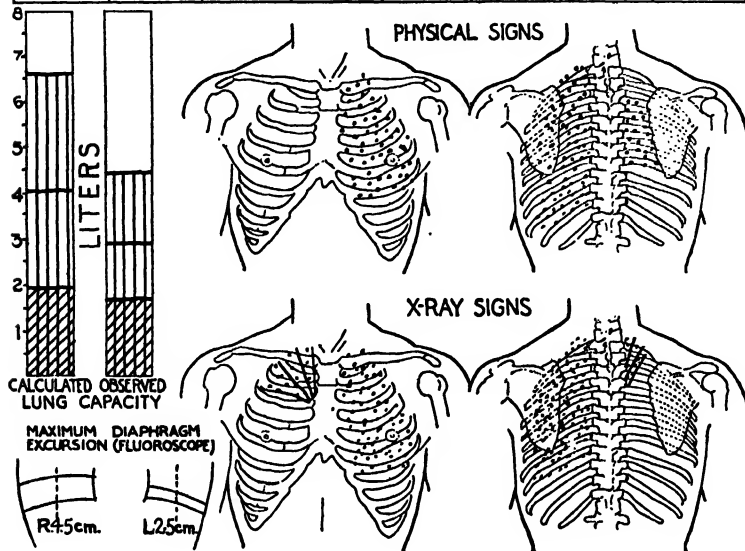
Height 175 cm.	Theoretical normal weight.....	kg. 70.0
Present weight.....		71.0
Patient's idea of normal weight.....		70.0
Date of highest weight 0 months ago.....		71.0
" " lowest " 8 " ".....		63.5
Treatment duration 13 months.		

**Physical Signs.**—April 9, 1917. Slightly dull percussion note at right apex to second rib anteriorly and to third spine posteriorly. Breath sounds moderately harsh in the same area. Fine and medium râles on cough at right apex to the third rib anteriorly and to the fourth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right upper lobe moderately densely infiltrated to the third rib. Mediastinal contents normal.

# No.17 (CASE 4363)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	*CHEST VOLUME*			
REST	cm. 20.0	cm. 19.7	cm. 28.0	liters 11.04	liters 2.9	26.3	24.9
MAX. INSP.	20.0	20.7	29.1	12.06	4.45	36.9	—
MAX. EXP.	20.0	18.9	27.0	10.20	1.7	16.6	—



TEXT-FIG. 17.

No. 17 (Case 4363).—Male, sign writer; age 29 years. Moderately advanced; active. Sputum + on admission.

Present illness began 25 months ago with malaise and tendency to tire easily. Gastric symptoms. Loss of 4.5 kg. in weight. Later moderate cough with scanty expectoration. His physical condition remains good under sanatorium treatment; symptoms about the same. Lung condition about the same.

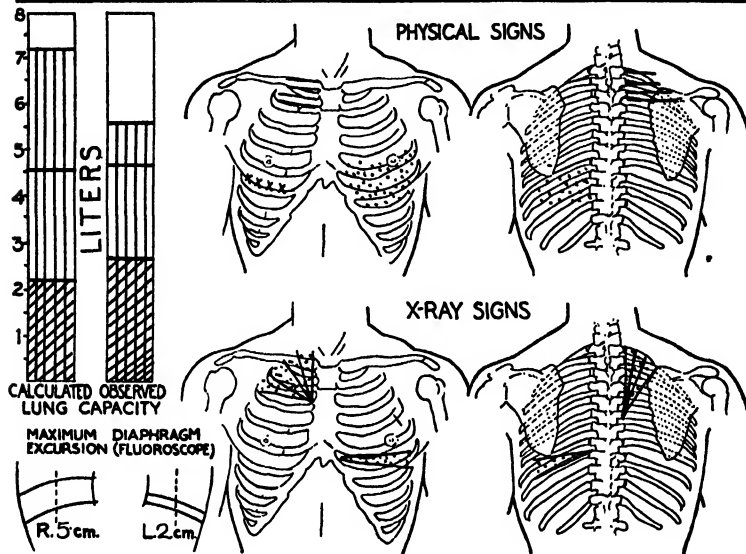
Height 174 cm.	Theoretical normal weight.....	kg. 70.0
Present weight.....		69.0
Patient's idea of normal weight.....		70.0
Date of highest weight 19 months ago.....		75.5
" " lowest " 17 " " .....		60.0
Treatment duration 1 month.		

**Physical Signs.**—April 7, 1917. No marked percussion changes. Increased breath sounds at right apex posteriorly. Fine râles on cough posteriorly from spine to angle of scapula. Medium and coarse râles, on cough, in left lung anteriorly and posteriorly from apex to base.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces slightly infiltrated. Rest of lung normal. Entire left lung slightly infiltrated, with fine spotting. Mediastinal contents completely to the left. Right lung area large.

# No. 18 (CASE 3997)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP	RATIO 100 X VITAL CAP
	STERNUM	ANT. POST.	TRANSVERSE	CHEST VOLUME*		CHEST VOL.	CHEST VOL.
REST	cm. 22.0	cm. 19.1	cm. 28.9	liters 12.2	liters 4.62	37.7	24.2
MAX. INSP.	22.0	19.9	29.9	13.1	5.57	42.5	—
MAX. EXP.	22.0	18.3	28.6	11.5	2.62	22.8	—



TEXT-FIG. 18.

No. 18 (Case 3997).—Male, machinist; age 27 years. Moderately advanced; inactive. Sputum — = +, on admission, in course of treatment, and at present.

Onset 16 months ago with moderate hemoptysis; later slight cough, occasional night sweat, and loss of 2 kg. in weight. Has felt well during his entire stay in the hospital.

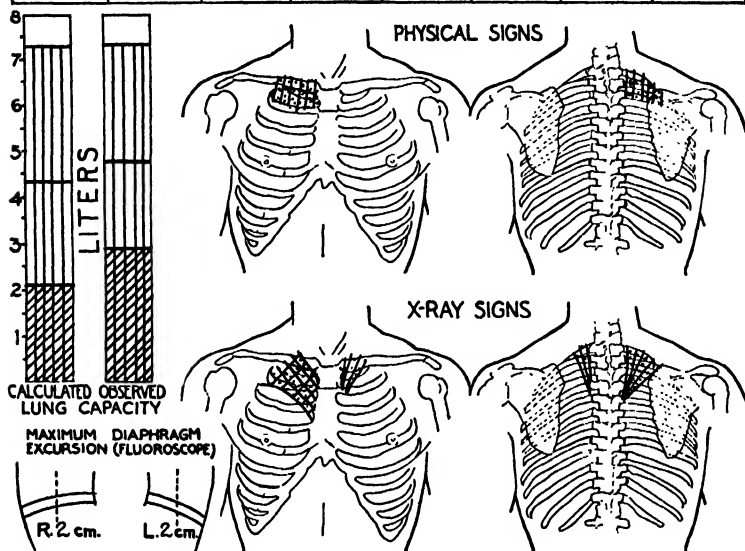
Height 174 cm.	Theoretical normal weight.....	kg. 70.0
Present weight.....		76.5
Patient's idea of normal weight.....		66.0
Date of highest weight 6 months ago.....		79.0
" " lowest " ? " " .....		70.0
Treatment duration 12 months.		

*Physical Signs.*—April 9, 1917. Moderate dullness at right apex, anteriorly to the second rib, posteriorly to the spine of the scapula. Breath sounds slightly increased at the left base. Fine moist râles on cough at left base, anteriorly below the fourth rib, posteriorly below a point midway between the spine and the angle of the scapula. Friction rubs at right base anteriorly.

*X-Ray Signs.*—April 7, 1917. Right apex and first and second interspaces slightly stippled and striated. Left, fifth interspace moderately spotted and striated. Mediastinal contents normal.

# No. 19 (CASE 4268)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP	RATIO 100 X VITAL CAP.
	STERNUM	ANTE. POST.	TRANSVERSE	CHEST VOLUME		CHEST VOL.	CHEST VOL.
REST	cm. 20.0	cm. 20.0	cm. 29.7	liters 11.9	liters 4.78	40.2	38.2
MAX. INSP.	20.0	21.5	31.2	13.4	7.38	55.0	—
MAX. EXP.	20.0	19.5	28.6	11.1	2.93	26.4	—



TEXT-FIG. 19.

No. 19 (Case 4268).—Male, teamster; age 29 years. Moderately advanced; inactive. Sputum + ± —, on admission, in course of treatment, and at present.

Present illness began 14 months ago after an attack of supposed influenza. Malaise; weakness; loss of 6.8 kg. in weight; 5 months after onset afternoon fever; occasional chills. Shortly after began to cough; expectoration profuse. Slight pain in left base. Under sanatorium treatment his symptoms have largely disappeared, his general condition is excellent, and the lung condition is apparently greatly improved.

Height 186 cm.	Theoretical normal weight.....	kg. 78.0
Present weight.....		87.0
Patient's idea of normal weight.....		84.0
Date of highest weight 3-months ago.....		92.0
“ “ lowest “ 10 “ “.....		79.5

Treatment duration 5 months.

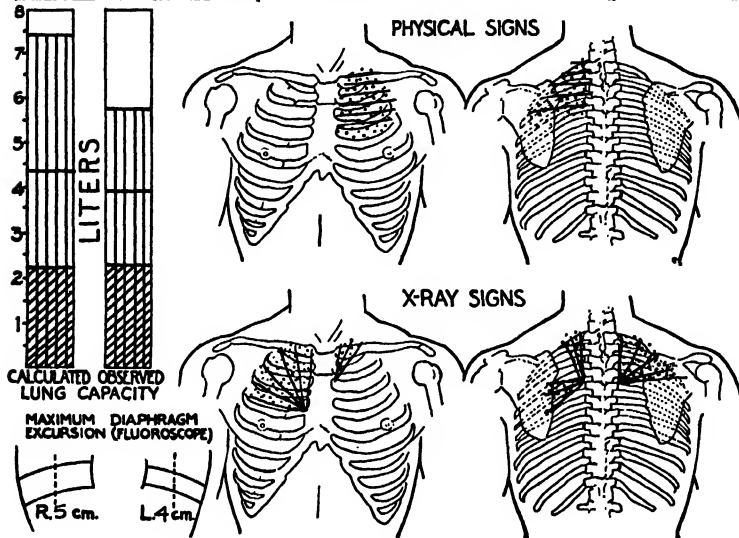
**Physical Signs.**—April 9, 1917. Slight dullness on percussion at right apex to the second rib anteriorly and the third dorsal spine posteriorly. Breath sounds slightly harsh. Fine râles on cough at apex to second rib anteriorly and to third spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces densely infiltrated. The lower edge of the infiltration is sharply limited from healthy lung below. Left apex infiltrated. Mediastinal contents normal.



# No. 20 (CASE 4006)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.2	19.8	28.5	11.95	3.90	32.5	29.3
MAX. INSP.	21.2	21.1	30.2	13.55	5.80	42.7	—
MAX. EXP.	21.2	19.6	28.2	11.70	2.30	19.7	—



TEXT-FIG. 20.

No. 20 (Case 4006).—Male, conductor on elevated railroad; age 33 years. Moderately advanced; inactive. Sputum — — —, on admission, in course of treatment, and at present.

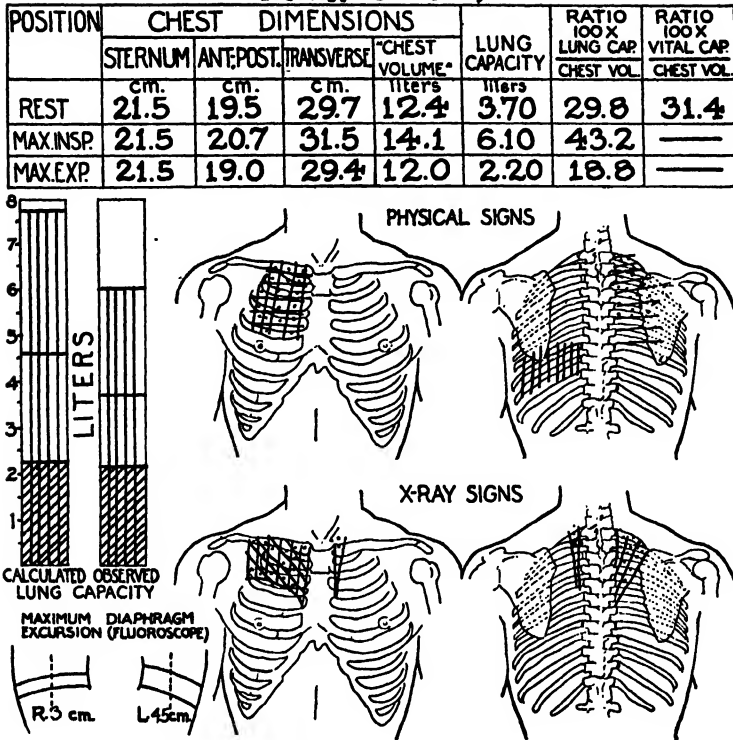
Present illness began 27 months ago with bronchitis. Severe cough for 2 months. Fever; night sweats; loss of 5.4 kg. in weight. Moderate weakness. Hemoptysis of moderate amount 6 months after onset. Pain at left base. Under sanatorium treatment his symptoms remain about the same except improvement in strength. No fever since onset. His general physical condition remains excellent.

Height 166 cm.	Theoretical normal weight.....	kg. 64.5
Present weight.....		77.0
Patient's idea of normal weight.....		84.0
Date of highest weight 4 years ago.....		84.0
“ “ lowest “ 8 months “ .....		75.0
Treatment duration 11 months.		

*Physical Signs.*—April 9, 1917. No change in percussion over right lung. Breath sounds slightly harsh at right apex. Moderate dullness at left apex to third rib anteriorly and to fourth spine posteriorly. Breath sounds feeble in the same area. Fine and medium râles on cough at left apex to fourth rib anteriorly and to fifth spine posteriorly.

*X-Ray Signs.*—April 7, 1917. Right apex and first three interspaces moderately densely striated and spotted. Left apex slight spotting. Mediastinal contents normal.

# No. 21 (CASE 4076)



TEXT-FIG. 21.

No. 21 (Case 4076).—Male, farmhand; age 27 years. Moderately advanced; inactive. Sputum — —, on admission, in course of treatment, and at present.

Onset 34 months ago with fever; loss of 24.5 kg. in weight in the first 6 months. Chills; night sweats; cough and expectoration slight. Slight hemoptysis. General condition during his hospital stay has been excellent; lung condition improved.

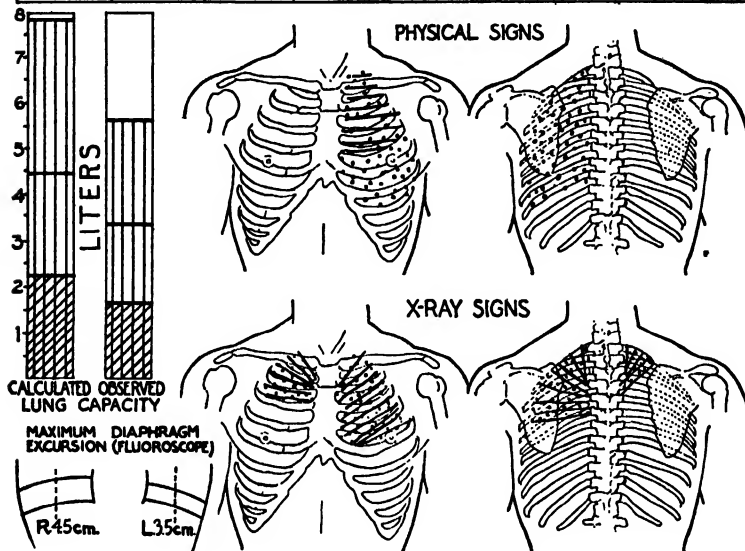
Height 183 cm.	Theoretical normal weight.....	kg. 77.0
Present weight.....		89.0
Patient's idea of normal weight.....		86.0
Date of highest weight 12 months ago.....		97.0
" " lowest " 34 " ".....		61.5
Treatment duration 10 months.		

**Physical Signs.**—April 9, 1917. Marked dullness of right lung anteriorly to the fourth rib, posteriorly moderate dullness from apex to spine of the scapula. Slight dullness at the left base below angle of scapula. No great change in breath sounds. Medium moist râles on cough at right apex to the third rib. Scattered fine moist râles on cough at right apex posteriorly to midway between the spine and angle of the scapula.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. The lower border of this infiltration has a sharp convex line, convexity upward. Left apex moderately densely infiltrated. Mediastinal contents normal.

# No. 22 (CASE 4082)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 23.8	cm. 19.0	cm. 26.7	liters 12.08	liters 3.34	27.6	32.7
MAX. INSP.	23.8	20.8	28.8	14.25	5.59	39.3	—
MAX. EXP.	23.8	18.8	25.9	11.06	1.64	14.1	—



TEXT-FIG. 22.

No. 22 (Case 4082).—Male, butcher; age 22 years. Moderately advanced; active. Sputum + = +, on admission, in course of treatment, and at present.

Onset 13 months ago with cold. Cough and expectoration moderate; slight pain in left side; slight dyspnea. During his stay in the sanatorium his general condition has remained good and his lung condition is apparently much improved.

Height 181 cm.	Theoretical normal weight.....	kg. 72.5
Present weight.....		74.5
Patient's idea of normal weight.....		76.0
Date of highest weight 22 months ago.....		78.0
" " lowest " 8 " ".....		73.0

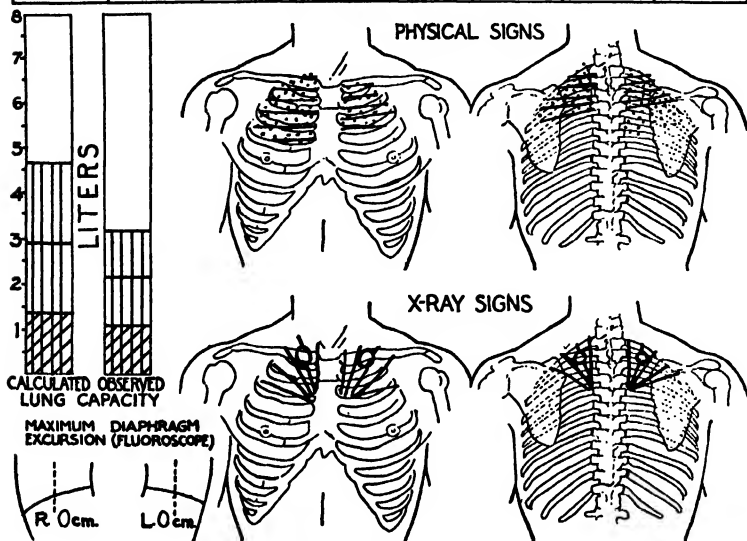
Treatment duration 10 months.

**Physical Signs.**—April 9, 1917. Dullness of upper part of left lung anteriorly to the fourth rib. No marked change in breath sounds. Coarse râles on cough in left lung both anteriorly and posteriorly to the base.

**X-Ray Signs.**—April 7, 1917. Right apex moderately densely infiltrated. First and second interspaces slightly stippled. Left apex and first and second interspaces very densely infiltrated. Third and fourth interspaces moderately spotted. Mediastinal contents to the left. Right lung area greatly increased.

# No. 23 (CASE 4911)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	18.9	17.4	23.6	7.8	2.1	26.9	27.0
MAX. INSP.	18.9	18.0	24.4	8.3	3.2	38.6	—
MAX. EXP.	18.9	17.1	23.3	7.5	1.1	14.7	—



TEXT-FIG. 23.

No. 23 (Case 4911).—Male, factory worker; age 19 years. Advanced; inactive. Sputum +++, on admission, in course of treatment, and at present.

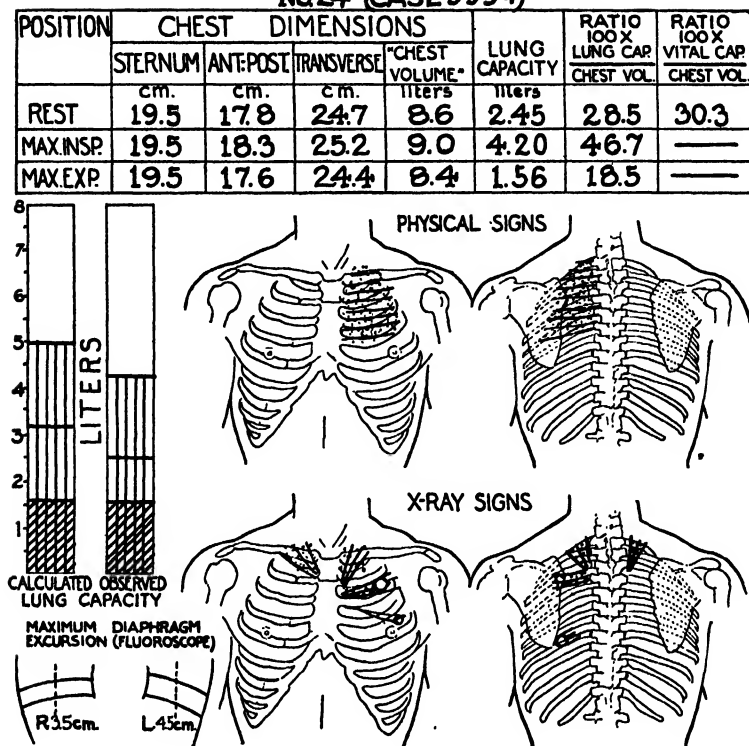
Onset 17 months ago with symptoms of grippe; began to cough slightly, although weight was not lost. Diagnosis made by sputum examination. Improved rapidly under sanatorium treatment and gained weight. Cough and expectoration markedly diminished.

Height 163 cm.	Theoretical normal weight.....	57.0
Present weight.....		53.0
Patient's idea of normal weight .....		46.0
Date of highest weight 5 months ago.....		54.5
" " lowest " 11 " .....		45.3
Treatment duration 11 months.		

**Physical Signs.**—April 9, 1917. Right, moderate dullness, very harsh breathing. Numerous râles on cough to fourth rib anteriorly, and sixth spine posteriorly. Left, moderate dullness, harsh breathing. Râles on cough to third rib anteriorly, and fourth spine posteriorly. Signs of cavity on both sides anteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex, cavity 4 cm. in diameter. First and second interspaces densely striated. Left apex, cavity 6 cm. in diameter. First and second interspaces densely infiltrated. Mediastinal contents normally placed.

# No 24 (CASE 3334)



TEXT-FIG. 24.

No. 24 (Case 3334).—Male, student; age 16 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 44 months ago with an acute cold, cough, and fever, soon followed by night sweats. On admission 32 months ago improved rapidly in general condition and weight, and is now with minimum cough and expectoration and in excellent general condition.

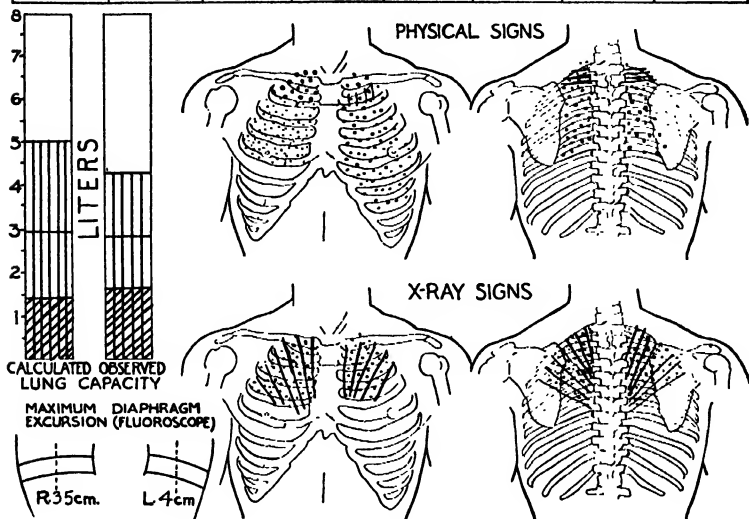
Height 165 cm.	Theoretical normal weight.....	kg. 59.0
Present weight.....		63.0
Patient's idea of normal weight.....		59.0
Date of highest weight 29 months ago.....		63.5
" " lowest " 3 years ".....		52.0
Treatment duration 32 months.		

*Physical Signs.*—April 9, 1917. Right lung clear. Left, moderate dullness, slight harsh breathing, and numerous fine râles on cough anteriorly to fourth rib and posteriorly to sixth spine.

*X-Ray Signs.*—April 7, 1917. Right apex very slightly stippled Left apex densely stippled and striated. Second interspace densely infiltrated and stippled, with cavity 3 by 2 cm. In the fourth interspace is a very small cavity the size of a bean. Mediastinal contents normally placed.

# **No. 25 (CASE 4300)**

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE. POST.	TRANSVERSE	CHEST VOLUME*			
REST	cm. 19.2	cm. 17.0	cm. 24.5	liters 8.0	liters 2.86	35.8	33.7
MAX INSP.	19.2	18.2	26.0	9.1	4.31	47.4	—
MAX EXP.	19.2	16.3	24.3	7.6	1.61	21.2	—



**TEXT-FIG. 25.**

**No. 25 (Case 4300).**—Male, machinist; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 17 months ago with malaise, weakness, and loss of 9 kg. in weight. Later severe cough, moderate expectoration, and night sweats. Fever, 100–101° F. During his stay in the sanatorium the fever has subsided and cough improved; no change otherwise.

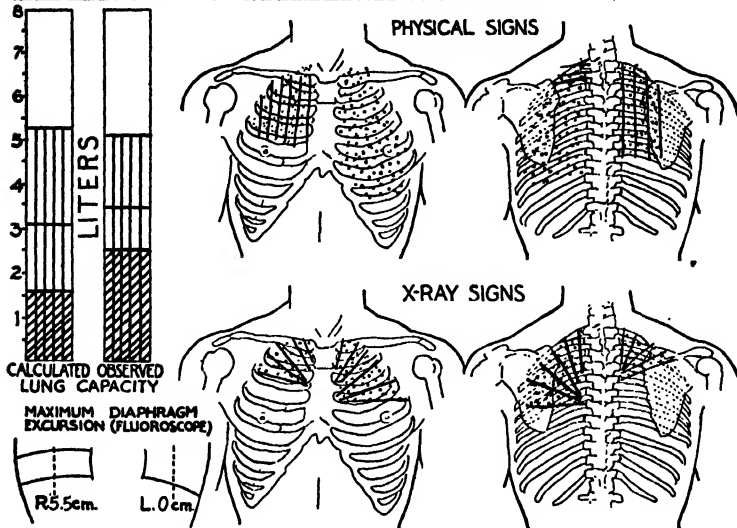
Height 173 cm.	Theoretical normal weight.....	66.5
Present weight.....		52.0
Patient's idea of normal weight.....		58.0
Date of highest weight 21 months ago.....		59.0
“ “ lowest “ 10 “ “.....		47.5
Treatment duration 3 months.		

**Physical Signs.**—April 9, 1917. Anteriorly slight dullness in the first interspace on the left side. Dullness at both apices posteriorly. The breath sounds are increased at the right apex anteriorly and posteriorly. Coarse râles on cough at right apex to the second rib; below this fine râles to the fifth rib. Posteriorly coarse râles at upper part of right lung to the angle of the scapula. Coarse râles on cough in left lung to the base anteriorly and to the angle of the scapula posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace densely infiltrated. Second and third interspaces slightly infiltrated and spotted. Rest of lung normal. Left apex and first, second, and third interspaces densely infiltrated and spotted. Mediastinal contents centrally placed. Trachea a little to the right.

# No. 26 (CASE 4127)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTEPOST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 18.6	cm. 17.0	cm. 27.4	liters 8.66	liters 3.45	39.7	30.1
MAX. INSP.	18.6	17.9	29.1	9.67	5.10	52.8	—
MAX. EXP.	18.6	16.4	27.0	8.23	2.50	30.4	—



TEXT-FIG. 26.

No. 26 (Case 4127).—Male, customs inspector; age 24 years. Advanced; active. Sputum + ± —, on admission, in course of treatment, and at present.

Onset 37 months ago with slight cough and expectoration. Loss of 3 kg. in weight. Sputum blood-streaked. His general condition during his hospital stay has remained good. He has had several slight hemoptyses. His lung condition apparently is unchanged.

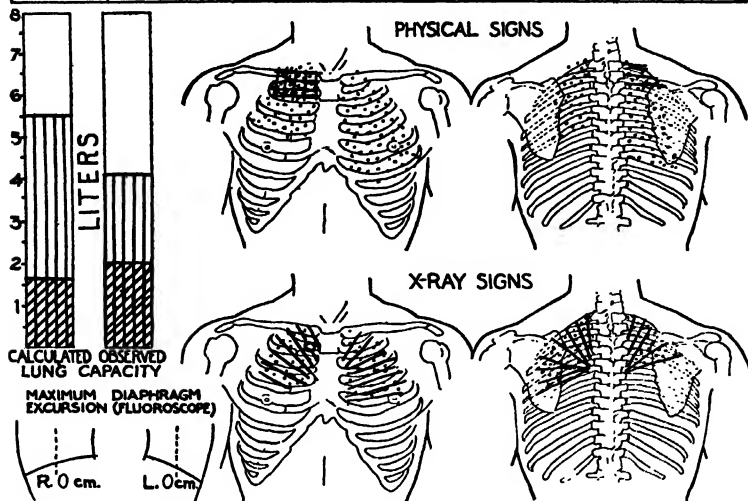
Height 168 cm.	Theoretical normal weight.....	63.0
Present weight.....		61.0
Patient's idea of normal weight.....		60.0
Date of highest weight 12 months ago.....		65.5
" " lowest " 18 " ".....		56.5
Treatment duration 8 months.		

**Physical Signs.**—April 9, 1917. Slight dullness over right lung, anteriorly to the fourth rib and posteriorly to the angle of the scapula. Dullness at left apex anteriorly. Harsh breath sounds at right apex. Slightly increased breath sounds at left apex posteriorly. Fine moist râles on cough at upper part of right lung to the third rib anteriorly and to the angle of the scapula posteriorly. Medium moist râles on cough in left lung to the base, both anteriorly and posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left apex and first, second, and third interspaces quite densely stippled and infiltrated. Mediastinal contents are entirely to the left. Right lung area much increased.

# No. 27 (CASE 437)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY liters	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM cm.	ANTEPOST cm.	TRANSVERSE cm.	CHEST VOLUME liters			
REST	20.0	17.0	27.2	9.25	—	—	22.7
MAX. INSP.	20.0	17.5	28.6	10.04	4.15	41.3	—
MAX. EXP.	20.0	16.7	26.2	8.75	2.05	23.4	—



TEXT-FIG. 27.

No. 27 (Case 437).—Male, laborer; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 12 months ago with severe cough. Later fever and chills. Slight pain in chest. Tendency to tire easily. His general condition has remained poor under sanatorium treatment with the lung condition progressive.

Height 178 cm.	Theoretical normal weight.....	70.5
Present weight.....		59.0
Patient's idea of normal weight.....		71.5
Date of highest weight 11 months ago.....		71.5
“ “ lowest “ 8 “ “ .....		57.5
Treatment duration 3 months.		

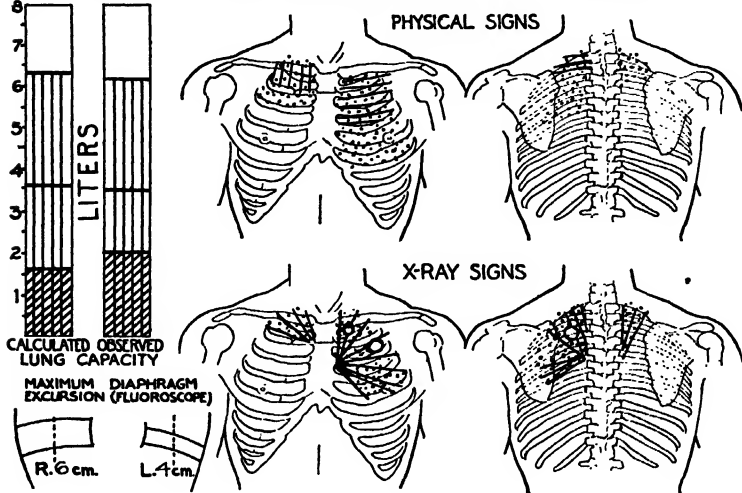
**Physical Signs.**—April 9, 1917. Marked dullness at right apex to the second rib. Moderate dullness at right apex posteriorly. Harsh breath at right apex to the second rib. Increased breath sounds at left apex to the third rib. Breath sounds increased at both apices posteriorly. Medium moist râles on cough in right lung from apex to fourth rib anteriorly, and from apex to below the angle of the scapula posteriorly. Medium moist râles on cough at left apex to the second rib anteriorly; below this fine râles to the base. Posteriorly medium moist râles on cough from the apex to an inch above the angle of the scapula.

**X-Ray Signs.**—April 7, 1917. Right apex perfectly clear. First, second, and third interspaces densely infiltrated. The apex may be excavated, but does not give physical signs of cavity. There is a small cavity 1 cm. in diameter at the inner end of the first interspace. Left apex and first and second interspaces densely infiltrated and spotted. Third and fourth interspaces slightly infiltrated and spotted. Mediastinal contents normally placed.



**No. 28 (CASE 4346)**

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE. POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	19.9	18.3	26.5	9.65	3.5	36.2	4:4.1
MAX. INSP.	19.9	18.7	28.5	11.60	6.2	53.5	—
MAX. EXP.	19.9	17.1	25.3	8.60	2.0	23.3	—



**TEXT-FIG. 28.**

**No. 28 (Case 4346).—**Male, pattern maker; age 31 years. Advanced; inactive. Sputum + on admission.

Onset 31 months ago with fever, chills, and night sweats. Cough and expectoration moderate. Rectal fistula. Anorexia, hoarseness, and return of fever 5 months ago. Loss of 4.5 kg. in weight since. During his stay in the hospital his general condition has been unchanged and his lung condition about the same.

Height 174 cm.	Theoretical normal weight.....	kg.	69.0
Present weight.....			61.5
Patient's idea of normal weight.....			62.5
Date of highest weight 14 months ago.....			67.0
“ “ lowest “ 1 month “.....			58.0

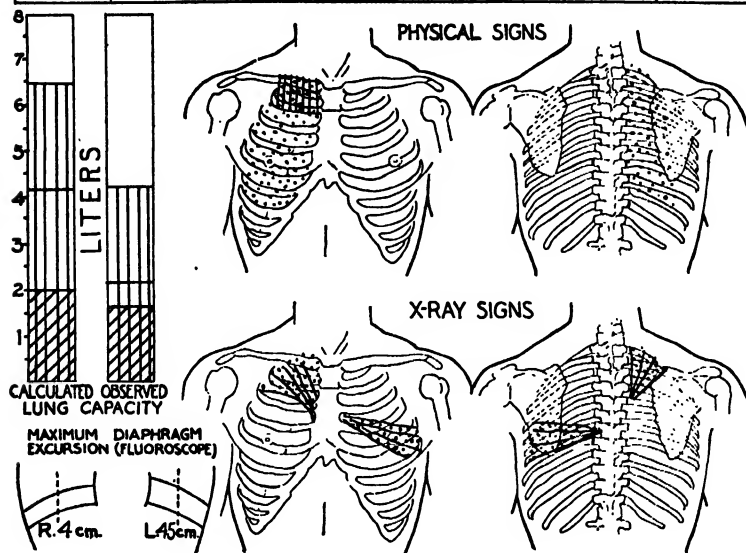
**Treatment duration 1 month.**

*Physical Signs.*—April 9, 1917. Slight dullness at right apex to the second rib. Dullness at upper part of left lung from the second to the fourth ribs. Left apex dull posteriorly. The breath sounds are increased from the clavicle to the fourth rib on the left side. Medium moist râles on cough at the right apex to the third rib. Medium moist râles on cough at the right apex posteriorly. Medium moist râles on cough from the clavicle to the sixth rib on the left side, and posteriorly from the apex to a point midway between the spine and angle of the scapula.

**X-Ray Signs.**—April 7, 1917. Right, moderate infiltration, spotting, and striation of the apex and first interspace. Left, moderate spotting and striation of the apex. Slight spotting of the first interspace. Dense spotting in the second interspace; cavity 3 by 5 cm. in the outer half. Third interspace moderately spotted and striated. Fourth interspace densely spotted and striated. Small cavity under clavicle. Mediastinal contents markedly to the left. Right lung area greatly increased.

# No. 29 (CASE 3952)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	21.3	19.3	27.2	11.2	2.14	19.1	23.2
MAX. INSP.	21.3	19.6	28.2	11.8	4.24	35.9	—
MAX. EXP.	21.3	18.7	26.4	10.5	1.64	15.6	—



TEXT-FIG. 29.

No. 29 (Case 3952).—Male, jeweler; age 20 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 16 months ago with cough; severe from the beginning and unaccompanied by other symptoms except slight expectoration later. His general condition under sanatorium treatment has remained good; lung disease increased.

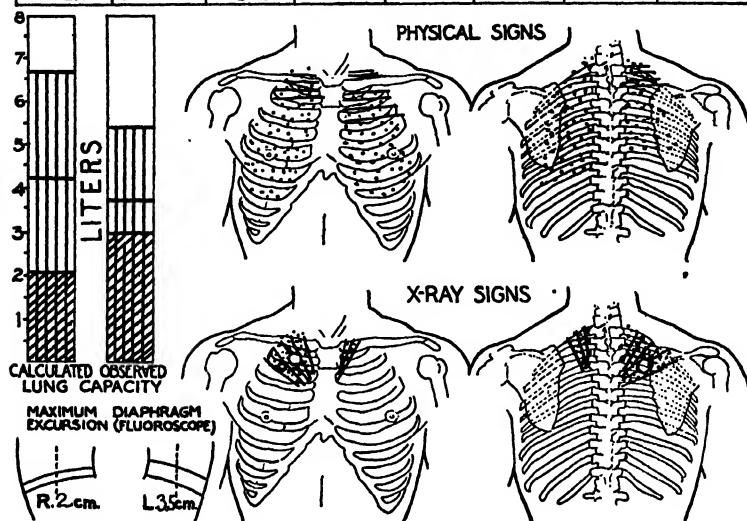
Height 174 cm.	Theoretical normal weight.....	66.0	kg.
Present weight.....		77.0	
Patient's idea of normal weight.....		68.0	
Date of highest weight 4 months ago.....		78.0	
" " lowest " 18 " ".....		68.0	
Treatment duration 12 months.			

**Physical Signs.**—April 9, 1917. Impaired resonance on percussion over the right apex anteriorly to the second rib. Diminished breath sounds over the entire left lung, anteriorly and posteriorly. Coarse râles on cough in right lung anteriorly from apex to base. Posteriorly fine râles on cough from apex to angle of scapula. Coarse râles on cough in left lung from the clavicle above to the sixth rib below. None heard posteriorly.

**X-Rays Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left, fifth and sixth interspaces moderately densely infiltrated. Mediastinal contents normal.

# No. 30(CASE 4027)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100% LUNG CAP CHEST VOL.	RATIO 100% VITAL CAP CHEST VOL.
	STERNUM	ANT:POST.	TRANSVERSE	"CHEST VOLUME" liters			
REST	cm. 21.4	cm. 20.2	cm. 26.0	liters 11.55	liters 3.7	32.1	21.2
MAX. INSP.	21.4	20.6	27.4	12.10	5.4	44.6	—
MAX. EXP.	21.4	19.9	25.7	11.00	3.0	27.3	—



TEXT-FIG. 30.

No. 30 (Case 4027).—Male, farmer; age 27 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 24 months ago with cough. Expectoration began 7 months later. Several small hemoptyses; moderate dyspnea. General condition bettered by sanatorium treatment; lung condition slightly progressive.

Height 179 cm.	Theoretical normal weight.....	74.5
Present weight.....		66.0
Patient's idea of normal weight.....		65.5
Date of highest weight 5 months ago.....		68.0
" " lowest " 20 " ".....		61.0

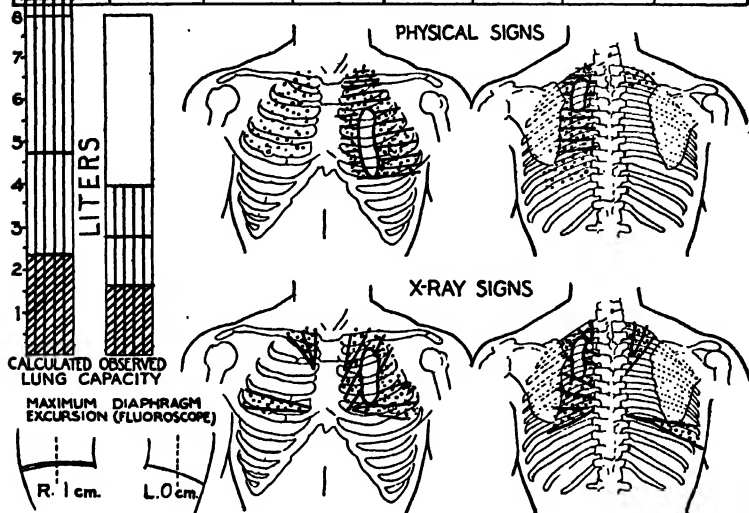
Treatment duration 11 months.

**Physical Signs.**—April 9, 1917. Moderate dullness over right apex anteriorly to the second rib, posteriorly to the spine. Moderate dullness over left lung anteriorly to the third rib; posteriorly no marked change. Breath sounds of upper part of right lung harsh at apex, bronchial in second interspace. Breath sounds increased in upper part of left lung to the third rib anteriorly. Coarse râles on cough in right lung to base anteriorly; posteriorly medium râles on cough from apex to angle of scapula. Medium râles on cough in left lung from clavicle above to the base; posteriorly fine râles on cough from apex to an inch below the angle of the scapula.

**X-Ray Signs.**—April 7, 1917. Right apex and first, second, and third interspaces very densely spotted and infiltrated. Moderate sized cavity in the first interspace. Left apex is moderately spotted. Mediastinal contents are normal.

# No. 31 (CASE 4130)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	22.0	19.7	29.3	12.70	2.82	22.2	18.9
MAX. INSP.	22.0	23.0	30.6	15.50	4.02	25.9	—
MAX. EXP.	22.0	19.3	28.6	12.15	1.62	13.3	—



TEXT-FIG. 31.

No. 31 (Case 4130).—Male, sheet metal worker; age 25 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

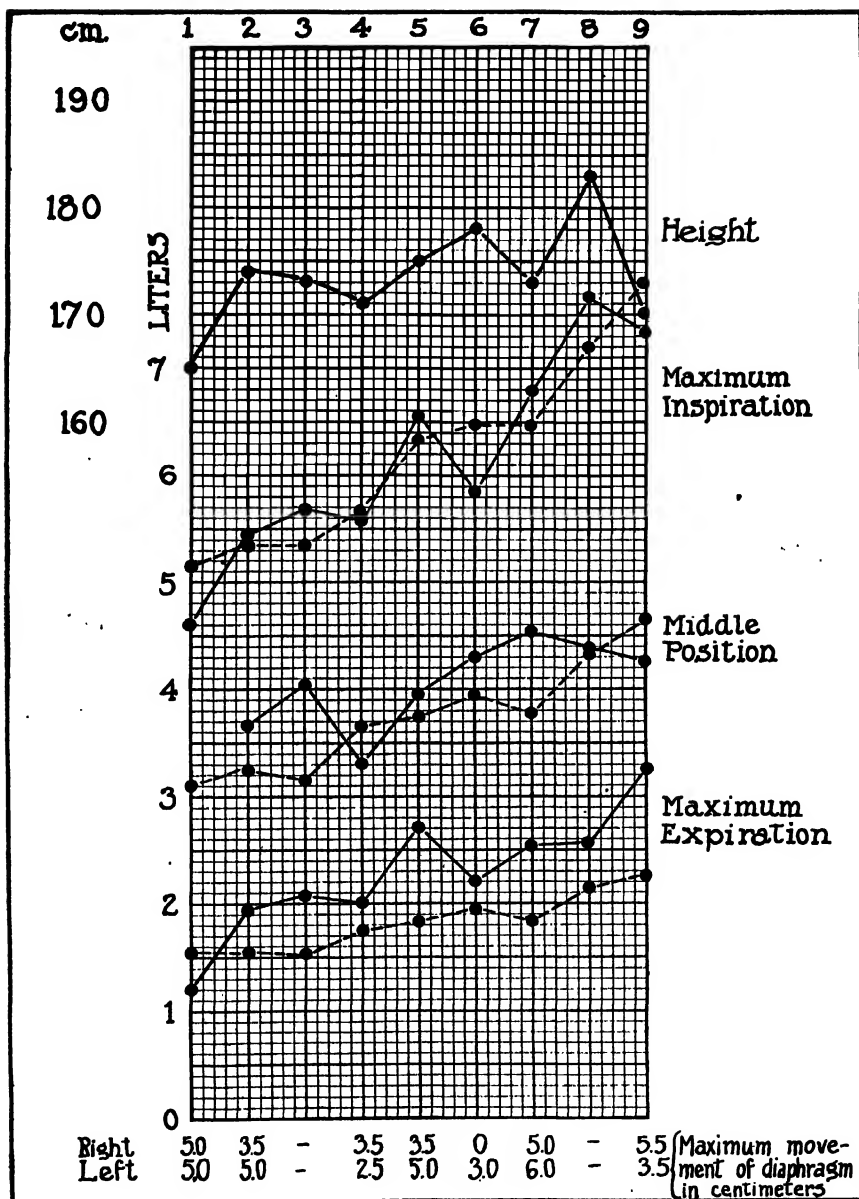
Onset 16 months ago with cold. Cough moderate; expectoration profuse; slight loss in weight; later chills and afternoon temperature of 100° F. Has been continuously toxic during stay in sanatorium; lung lesion progressive.

Height 177 cm. Theoretical normal weight.....	kg. 68.5
Present weight.....	?
Patient's idea of normal weight.....	72.5,
Date of highest weight 4 to 5 years ago.....	76.0
" " lowest " 12 months " .....	66.5
Treatment duration 8 months.	

**Physical Signs.**—April 9, 1917. Dullness anteriorly over the left lung from apex to the sixth rib. Cracked pot percussion in the second, third, fourth, and fifth interspaces. Dullness posteriorly over the left lung from the apex to the angle of the scapula. Breath sounds harsh posteriorly at right apex. No great change in right lung anteriorly. Diminished breath sounds in left lung posterior to base. Anteriorly slightly increased at left apex, cavernous in the third, fourth, and fifth interspaces.

**Rales:** Right apex to second rib on cough medium moist râles, second to fourth sibilant. Posteriorly medium moist on cough from apex to the spine of the scapula. Fine and medium moist râles on cough in left lung anteriorly and posteriorly from apex to base.

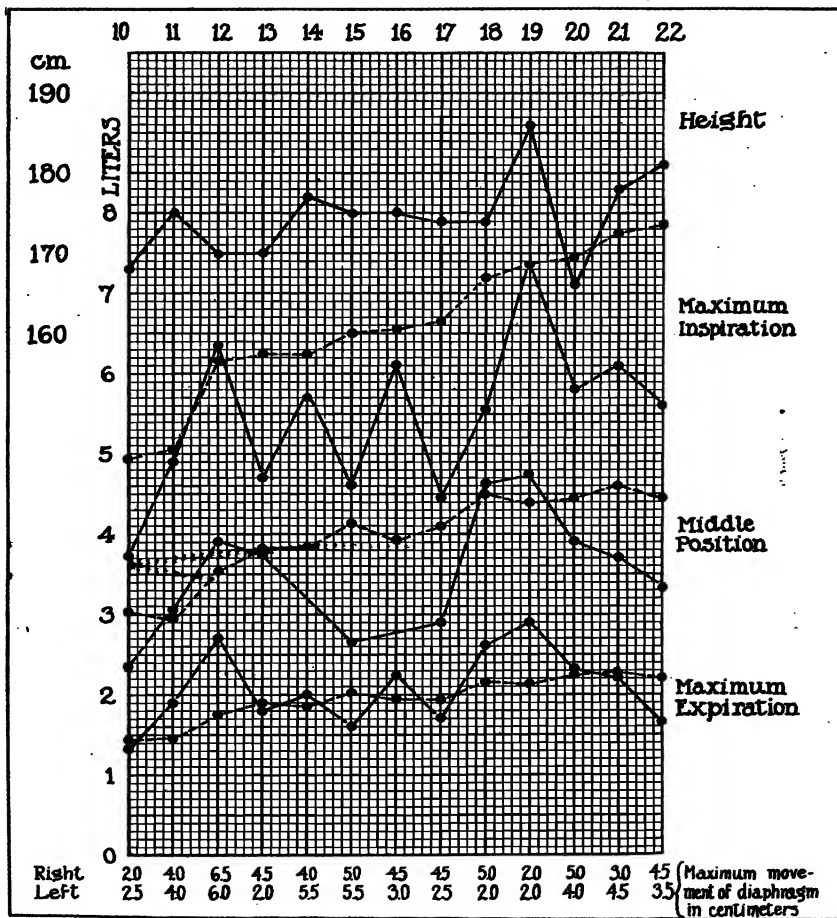
**X-Ray Signs.**—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Fourth interspace moderately densely infiltrated. Entire left lung densely spotted. Large cavity, 14 by 5 cm. Mediastinal contents markedly to the left. Right lung area greatly increased.



In the charts the numbers below indicate the maximum excursion of the right and left diaphragm. The numbers above the chart refer to the individual diagrams and descriptions (Text-figs. 1 to 31).

TEXT-FIG. 32. Lung volumes in men with incipient pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.

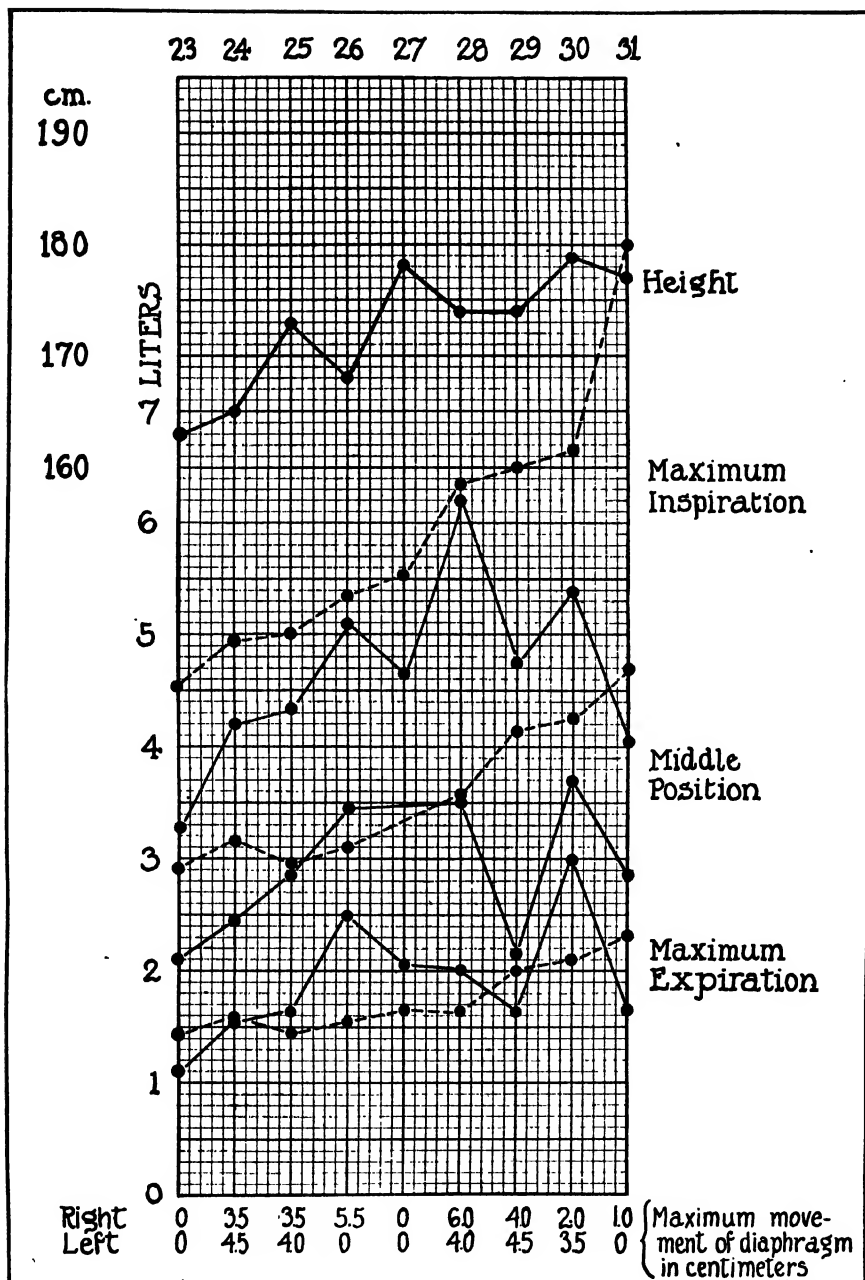




TEXT-FIG. 33. Lung volumes in men with moderately advanced pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.







TEXT-FIG. 34. Lung volumes in men with advanced pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



## STUDIES OF LUNG VOLUME.

### III. TUBERCULOUS WOMEN.

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(Received for publication, September 15, 1917.)

#### INTRODUCTION.

In the preceding paper (Garvin, Lundsgaard, and Van Slyke) a report was made of a series of determinations of the different lung volumes in thirty-one adult men suffering from pulmonary tuberculosis. A comparison was drawn between the actual values and the values calculated from the chest dimensions on the basis of certain ratios previously worked out on normal subjects (Lundsgaard and Van Slyke). The literature concerning pulmometry in pulmonary tuberculosis is given in the preceding paper.

The present paper is a report of similar determinations on twenty adult women with phthisis. The technique in determining the lung volumes, in measuring the chest wall, and in determining the movement of the diaphragm is fully described in Papers I and II. The presentation of the experimental and clinical observations on the women is carried out after exactly the same plan as in Paper II, where sufficient explanation can be found. Only the explanation of the symbols used in the individual diagrams (Text-figs. 1 to 20) will be repeated here:

#### *Physical Signs.*—

Light lines, slight dullness.

Heavy lines, moderate dullness.

Cross-hatching, marked dullness.

Fine dots, fine râles.

Larger dots, moderate and coarse râles

Small rings, large crackling râles.

Crosses, pleuritic rubs.

Circles, antrum formation.

There is no difference in the interpretation of horizontal and vertical lines.

*X-Ray Signs.*—

Lightly shaded lines, slight density of shadow.

Heavy lines, marked density.

Circles, cavity.

Dots, stippling, the larger the dots, the coarser the stippling.

There is no difference in the interpretation of horizontal and vertical lines.

The patients are divided into three groups, the incipient (Text-fig. 21), moderately advanced, and advanced cases. The last two groups, however, are described together and the values put together in Text-fig. 22. Nos. 9 and 19 are advanced (Group III in Paper II); the rest are moderately advanced (Group II in Paper II). The reason for this is that there is, as pointed out in Paper I, no sharp difference in our results between patients belonging to Groups II and III.

*Group I. Incipient Cases (Nos. 1 to 8, Text-Fig. 21).*

It will be remembered that the result of the determinations on nine men with incipient tuberculosis was (1) a normal total capacity, (2) a moderately increased residual air resulting in (3) a moderately diminished vital capacity. At first sight the results on the women seem quite opposite. The values in Nos. 1, 2, 3, 4, and 8 agree with those found in the men, but in Nos. 5, 6, and 7, although they also are clinically incipient, a great decrease in the total and vital capacity is encountered. A similar drop is seen in the middle capacity, whereas the residual air is normal.

However, sufficient cause can be found to account for this. No. 5 was a patient with miliary tuberculosis of the lungs. She had râles on both sides all over the lungs. No. 6 had a bronchial stenosis on the left side; the left lung participated only to a small extent in the ventilation. It will be seen that her left diaphragm moved in the opposite direction to the normal movement in respiration. This probably was a result of the difficult passage to the left lung. The observed vital and middle capacity is about half of the calculated. No. 7 did not move her diaphragm at all. The residual air, however,

is not increased, which probably can be looked upon as indicating that her diaphragm is fixed in expiratory position. The inability to lower the diaphragm at inspiration must, of course, diminish her total capacity to a considerable extent.

The results of the determinations in these cases therefore confirm the previous findings in men, and show that if the total capacity is diminished in patients with incipient tuberculosis, some special cause is to be found, such as miliary tuberculosis, obstruction of bronchi, or inability to move the diaphragm.

*Group II. Moderately Advanced and Advanced Cases (Nos. 9 to 20, Text-Fig. 22).*

Group II (Nos. 9 to 20, moderately advanced and advanced cases) shows the same picture that was found in men: (1) As a rule diminished total capacity. In all the cases except Nos. 9, 10, 13, and 17, the total capacity is below the normal minimum; in these four cases it is above the normal minimum but below the normal average. (2) Decreased vital capacity. (3) Fairly normal residual air. (4) The middle capacity (not determined in all cases) is in some patients normal, in others subnormal. As mentioned before, we do not lay much stress on the determination of the middle capacity, because it is dependent not only on anatomic but also on functional factors. What the latter are we do not understand, but we have seen subjects unconsciously inflate or deflate the chest so as to change the middle capacity by several hundred centimeters.

*Excursions of the Diaphragm.*

The technique is described in Papers I and II. The excursions of the diaphragm are, as a whole, smaller than in normal subjects and agree with those found in the men. In one instance (No. 7) no movement was found at all. No evidence was found for a mechanical obstacle in the pleura, lungs, or abdomen. Whether the diaphragm was paralyzed through involvement of the phrenic nerves, or whether it was due to a reflex, we do not know. In another case (No. 6) the left half of the diaphragm moved in the direction opposite to the normal. Sufficient explanation is found in the fact that the left

TABLE I.

*Influence of Change of Position and of Exercise on Pulse and Respiration.*

No. on individual diagrams.	Case No.	Resting in bed.		Standing up.		After having run up three flights of stairs.		
		Pulse.	Respirations.	Pulse.	Respirations.	Pulse.	Respirations.	Other symptoms.
Group I.								
1	4167	94	24	120	24	124	30	Very slight dyspnea. Pulse slows quickly.
2	4247	90	24	96	24	150	26	Slight dyspnea; cyanosis of hands.
3	4164	96	18	110	25	136	32	Palpitation; dyspnea; throbbing temples.
4	4215	74	18	90	24	150	44	Very nervous; very dyspneic; face flushed; hands cyanotic.
5	4190	102	30	112	34	150	42	Palpitations; dizziness.
6	4151	74	24	80	24	130	32	Very dyspneic; face slightly flushed.
7	4309	102	30	116	30	160	36	Slight dyspnea; face flushed.
8	3996	78	18	102	18	120	24	Face flushed.
Group II.*								
9	4061	80	17	115	18	145	22	None.
10	4314	72	18	96	20	132	28	Slight dyspnea; face slightly flushed.
11	4059	102	22	120	22	140	30	Slight dyspnea; face much flushed.
12	3882	72	26	84	24	150	30	Face flushed; dizziness; very dyspneic.
13	4191	72	16	102	16	160	20	None.
14	4044	90	24	110	22	147	26	Flush.
15	4192	72	22	84	22	150	30	Moderate dyspnea; face slightly pale.
17	4283	90	18	92	18	125	28	Palpitation; face flushed.
18	4264	90	12	100	16	120	24	"
19	3908	112	22	112	22	145	28	" marked dyspnea; face flushed; weak pulse.
20	4103	64	16	75	20	145	28	Slight dyspnea; face pale; pulse very weak.

\* Group II is composed of moderately advanced cases (Group II in Paper II) and advanced cases (Group III in Paper II).

main bronchus was almost obstructed. An inspiratory movement of the thoracic wall could, under these conditions, result in an upward movement of the diaphragm on this side.

*Influence of Change of Position and of Exercise on Pulse and Respiration*  
(Table I).

The increase in pulse rate on exercise is still more marked in the women than in the men. The rate of respiration is also somewhat increased. As a whole, in men no such increase in the respiration was found.

SUMMARY.

The total capacity, middle capacity, and residual air have been determined in twenty adult women suffering from pulmonary tuberculosis. The chest volumes have been determined in each case and the normal lung volumes calculated by means of the ratios worked out in Paper I and applied to thirty-one men in Paper II. The excursions of the diaphragm have been determined by fluoroscopy in all cases.

Of eight patients with incipient tuberculosis, five had lung capacities like those of men in the same group;<sup>1</sup> *i.e.*, about normal total capacity, slightly increased residual air, and consequently somewhat decreased vital capacity. Three had considerably diminished total capacity. In these three patients, however, clinical abnormalities were found (extensive miliary tuberculosis, obstruction of bronchus, fixation of diaphragm in expiratory position).

In twelve patients with moderately advanced and advanced tuberculosis, the results agreed with those found in men,<sup>1</sup> the total capacity and vital capacity being decreased, while the residual air was practically normal.

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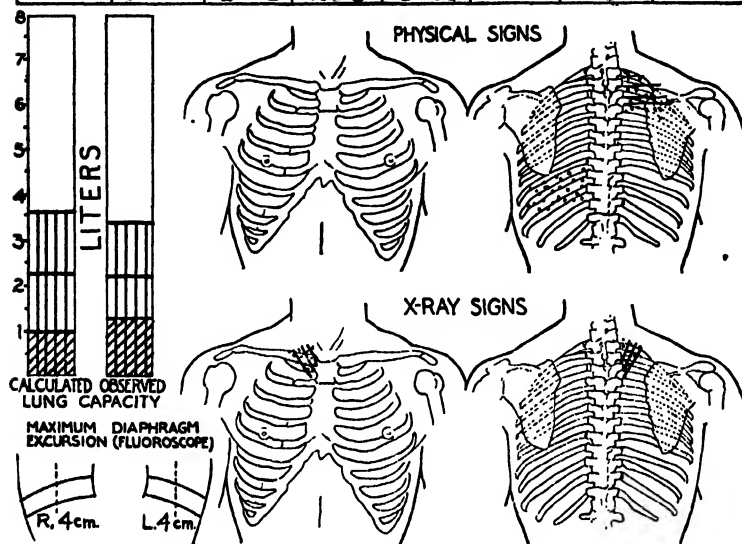
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<sup>1</sup> See Paper II.

<sup>2</sup> An extensive bibliography can be found in the two preceding papers.

# No.1 (CASE 4167)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	16.8	16.1	22.2	6.00	2.15	35.8	35.0
MAX. INSP.	16.8	17.3	22.6	6.56	3.35	51.0	—
MAX. EXP.	16.8	15.1	21.5	5.45	1.25	23.0	—



TEXT-FIG. 1.

No. 1 (Case 4167).—Female, factory worker; age 22 years. Incipient; active. Sputum — — +, on admission, in course of treatment, and at present.

Onset 17 months ago with slight cough, blood-streaked sputum, and loss of weight and strength. Under treatment has gained weight but disease is still slightly active. Physical signs have increased slightly in the past 7 months, and a previous negative sputum has become positive.

Height 157 cm.	Theoretical normal weight.....	kg. 53.5
Present weight.....		48.0
Patient's idea of normal weight.....		43.0
Date of highest weight 2 months ago.....		48.5
" " lowest " 12 " ".....		41.0

Treatment duration 7 months.

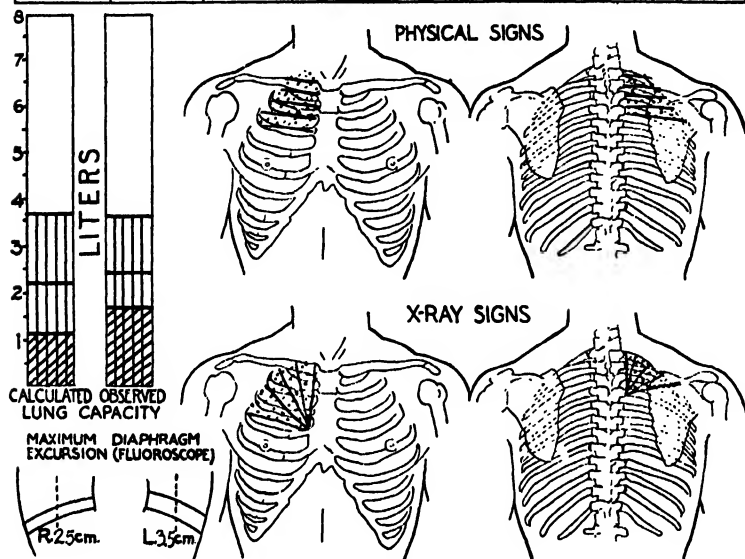
**Physical Signs.**—April 9, 1917. Right, no impairment of resonance; breathing harsh; fine râles with cough only posteriorly to fourth spine. Left, upper lobe normal; posteriorly there are moist râles from base to ninth dorsal spine with aid of cough; no dullness or breath sound change in this area, and signs are intrapulmonic (not affected by breathing and diaphragm has a normal excursion). (See illustration of normal volume.)

**X-Ray Signs.**—April 7, 1917. Right apex moderately densely infiltrated. Root slightly infiltrated. Left lung practically normal (see physical examination). Mediastinal contents normal.



# No. 2 CASE 4247)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE:POST.	TRANSVERSE	"CHEST VOLUME" liters			
REST	15.0	16.8	23.8	6.00	2.4	40.0	25.0
MAX. INSP.	15.0	17.4	25.5	6.65	3.6	54.2	—
MAX. EXP.	15.0	16.0	23.7	5.69	1.7	29.9	—



TEXT-FIG. 2.

No. 2 (Case 4247).—Female, student; age 18 years. Incipient; inactive. Sputum — ± +, on admission, in course of treatment, and at present.

Onset 16 months ago with cough and blood-streaked sputum. Under sanatorium treatment symptoms have disappeared and physical signs have diminished in number.

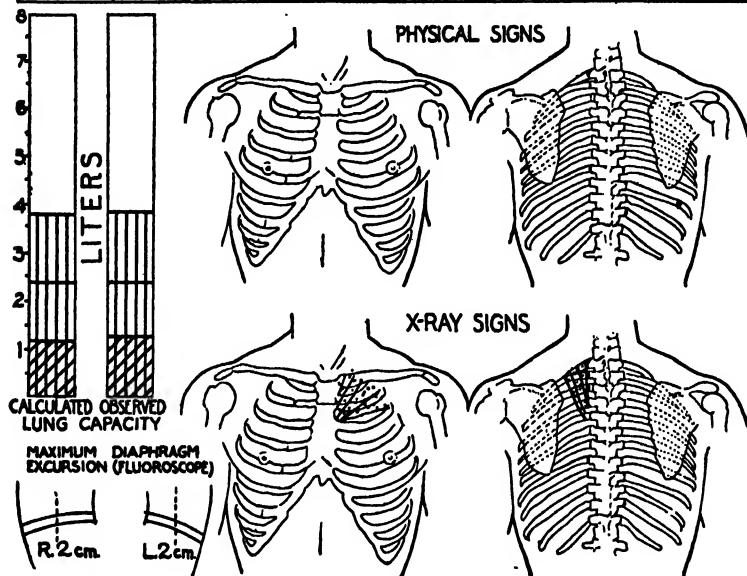
Height 156 cm.	Theoretical normal weight.....	52.0
Present weight.....		64.0
Patient's idea of normal weight.....		59.0
Date of highest weight 0 months ago.....		64.0
“ “ lowest “ 4 “ “ .....		58.0

Treatment duration 5 months.

*Physical Signs.*—April 9, 1917. Right, slight impairment of resonance; breath sounds are a little harsh; fine râles appear with aid of cough to the third rib anteriorly and fourth spine posteriorly. Left lung seems normal.

*X-Ray Signs.*—April 7, 1917. Right apex and first, second, and third interspaces very slightly stippled and striated. Left lung normal. Mediastinal contents are a little to the right. Left lung area increased.

No. 3 (CASE 4164)							
POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP	RATIO 100 X VITAL CAP
	STERNUM	ANTE. POST.	TRANSVERSE	CHEST VOLUME*		CHEST VOL.	CHEST VOL.
REST	cm. 15.8	cm. 15.3	cm. 26.4	liters 6.38	liters 2.40	36.6	41.5
MAX. INSP.	15.8	16.2	27.4	6.96	3.90	56.0	—
MAX. EXP.	15.8	14.9	25.7	6.06	1.25	20.6	—



TEXT-FIG. 3.

No. 3 (Case 4164).—Female, domestic; age 19 years. Incipient; inactive. Sputum + ± —, on admission, in course of treatment, and at present.

Onset 12 months ago with malaise and loss of strength; slight cough with streaked sputum for a few days. Under sanatorium treatment symptoms have diminished and physical signs have disappeared.

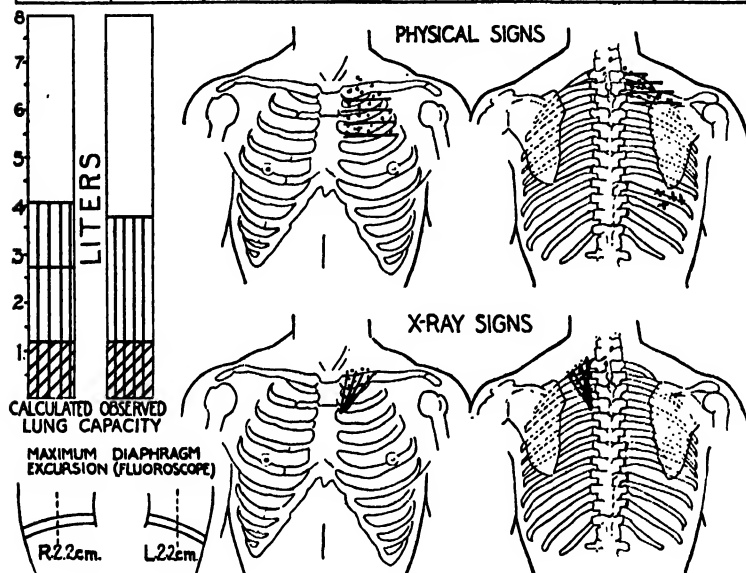
	kg.
Height 152 cm. Theoretical normal weight.....	51.0
Present weight.....	55.0
Patient's idea of normal weight.....	50.0
Date of highest weight 1 month ago.....	56.5
"    "    lowest    "    7 months    "    .....	47.5
Treatment duration 7 months.	

**Physical Signs.**—April 9, 1917. Right lung seems normal. Left lung seems normal. Patient has had tubercle bacilli in sputum within 6 weeks.

**X-Ray Signs.**—April 7, 1917. Right lung clear. Left apex stippled and striated moderately to the second rib. Mediastinal contents normal.

## No. 4 (CASE 4215)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP.	RATIO 100 X VITAL CAP.
	STERNUM	ANT. POST.	TRANSVERSE	*CHEST VOLUME		CHEST VOL.	CHEST VOL.
REST	cm. 16.7	cm. 17.2	cm. 23.4	liters 6.71	liters 2.52	37.0	36.0
MAX. INSP.	16.7	18.4	24.5	7.54	3.72	49.1	—
MAX. EXP.	16.7	16.3	22.5	6.12	1.22	19.9	—



TEXT-FIG. 4.

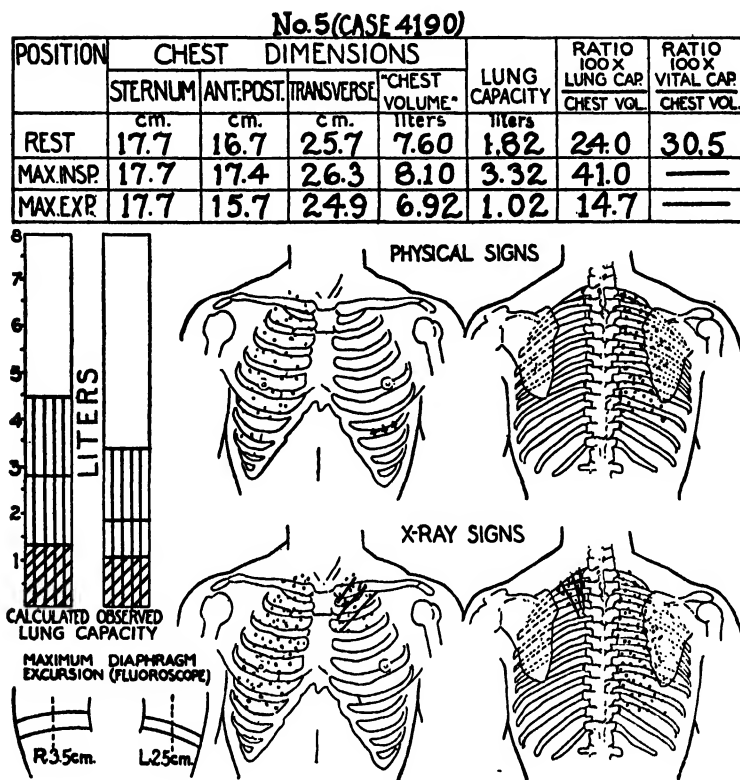
No. 4 (Case 4215).—Female, student; age 18 years. Incipient; inactive. Sputum ++, on admission, in course of treatment, and at present.

Onset 30 months ago with cough following influenza. Under sanatorium treatment symptoms have disappeared, and physical signs diminished about 75 per cent.

Height 155 cm.	Theoretical normal weight.....	kg. 51.0
Present weight.....		61.0
Patient's idea of normal weight.....		50.0
Date of highest weight 0 months ago.....		61.0
“ “ lowest “ 30 “ “ .....		47.0
Treatment duration 7 months.		

**Physical Signs.**—April 9, 1917. Left apex, slight dullness; breath sounds diminished; Râles fine and moist to third rib anteriorly and to spine of scapula posteriorly. Right base posteriorly gives fine friction rubs on deep breathing.

**X-Ray Signs.**—April 7, 1917. Right lung fairly normal. Left apex and first inter-space moderately densely infiltrated. Mediastinal contents normal.



TEXT-FIG. 5.

*No. 5 (Case 4190).*—Female, stock clerk; age 17 years. Incipient; inactive. Chronic miliary tuberculosis of the right lung. Sputum —, on admission and in course of treatment.

Onset 7 months ago with slight cough and malaise, and dyspnea on exertion. Under treatment has improved in symptoms while the signs have remained the same.

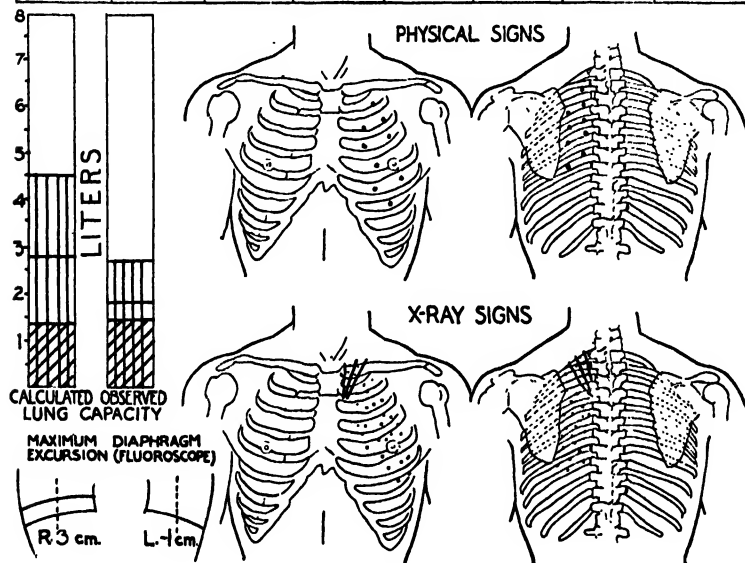
Height 158 cm.	Theoretical normal weight.....	53.5
Present weight.....		59.0
Patient's idea of normal weight.....		52.0
Date of highest weight 0 months ago.....		59.0
" " lowest " 17 " ".....		45.5

Treatment duration 6 months.

*Physical Signs.*—April 9, 1917. Right, no impairment of resonance or change in breath sounds; a few râles after cough fairly well disseminated throughout the right lung. Left, no change in resonance or in breath sounds; a few friction rubs at the base.

*X-Ray Signs.*—April 7, 1917. Right lung shows very fine discrete spotting throughout. Left apex and first and second interspaces show slight spotting and striations. Mediastinal contents normal.

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	CHEST VOLUME*			
REST	cm 18.2	cm. 17.6	cm. 23.5	liters 7.52	liters 1.86	24.7	18.0
MAX INSP.	18.2	18.0	24.7	8.15	2.74	33.6	—
MAX EXP.	18.2	16.9	22.9	7.04	1.41	20.2	—



TEXT-FIG. 6.

*No. 6 (Case 4151).*—Female, student; age 19 years. Incipient; inactive. Left primary bronchus obstruction (large gland). Sputum + + —, on admission, in course of treatment, and at present.

Onset 20 months ago with malaise, slight cough, and dyspnea. Under treatment she has gained much in weight, is still dyspneic, but feels much better. Physical signs unchanged.

	kg.
Height 158 cm. Theoretical normal weight.....	52.5
Present weight.....	52.5
Patient's idea of normal weight.....	54.5
Date of highest weight 22 months ago.....	56.5
" " lowest " 7 " ".....	48.5

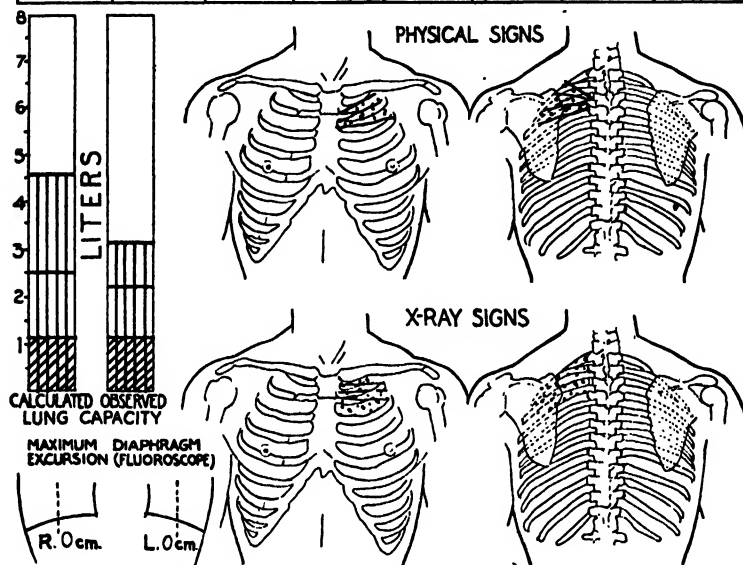
Treatment duration 7 months.

*Physical Signs.*—April 9, 1917. Right, chest normal; no impairment of resonance. Left, breathing diminished; coarse loud râles throughout left chest.

*X-Ray Signs.*—April 7, 1917. Right lung a little hazy throughout. Left lung, apical space slightly infiltrated; rest of the lung shows very fine slight spotting on full inspiration. Mediastinal contents are to left. Right lung area much increased.

## No 7 (CASE 4309)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP.	RATIO 100 X VITAL CAP.
	STERNUM	ANT. POST.	TRANSVERSE	CHEST VOLUME - liters		CHEST VOL.	CHEST VOL.
REST	cm. 16.9	cm. 16.7	cm. 23.7	6.60	liters 2.20	33.0	29.5
MAX. INSP.	16.9	18.7	26.2	8.30	3.10	37.3	—
MAX. EXP.	16.9	15.5	23.1	6.04	1.15	19.0	—



TEXT-FIG. 7.

No. 7 (Case 4309).—Female, student; age 16 years. Incipient; inactive. Sputum + + —, on admission, in course of treatment, and at present.

Onset 8 months ago with malaise and slight cough. Under treatment symptoms and signs have markedly diminished. Patient feels perfectly well.

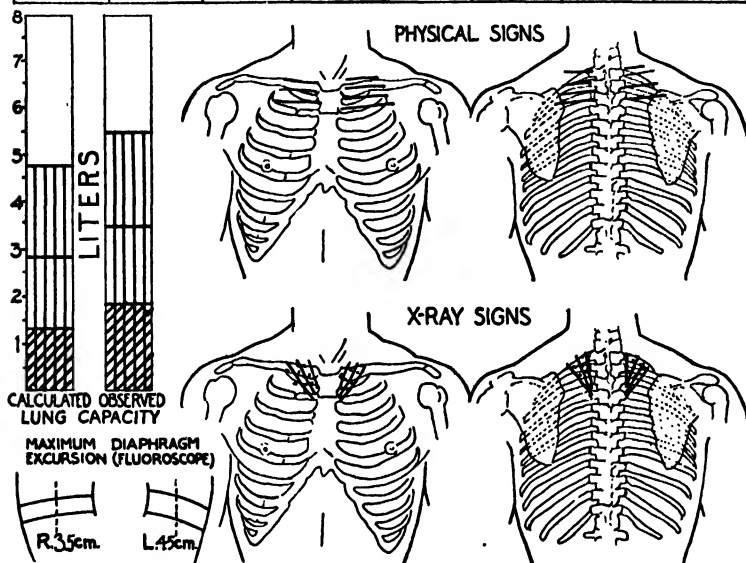
Height 157 cm.	Theoretical normal weight.....	kg. 52.0
Present weight.....		48.5
Patient's idea of normal weight....		45.5
Date of highest weight 0 months ago.....		48.5
" " lowest " 15 " ".....		45.5
Treatment duration 3 months.		

**Physical Signs.**—April 9, 1917. Right lung normal. Left, slight dullness below clavicle; breath sounds are a little harsh; râles over second and third interspaces anteriorly and to fourth spine posteriorly with aid of cough.

**X-Ray Signs.**—April 7, 1917. Right lung normal. Left, first and second interspaces moderately densely infiltrated. Mediastinal contents normal.

## No. 8 (CASE 3996)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 18.7	cm. 16.7	cm. 24.4	liters 7.61	liters 3.42	45.0	46.7
MAX. INSP.	18.7	18.5	25.1	8.68	5.42	62.2	—
MAX. EXP.	18.7	16.0	23.4	7.00	1.87	26.7	—



TEXT-FIG. 8.

*No. 8 (Case 3996).*—Female, trained nurse; age 35 years. Incipient; inactive. Sputum + = —, on admission, in course of treatment, and at present.

Onset 25 months ago with malaise and cough. Under sanatorium treatment general condition much improved.

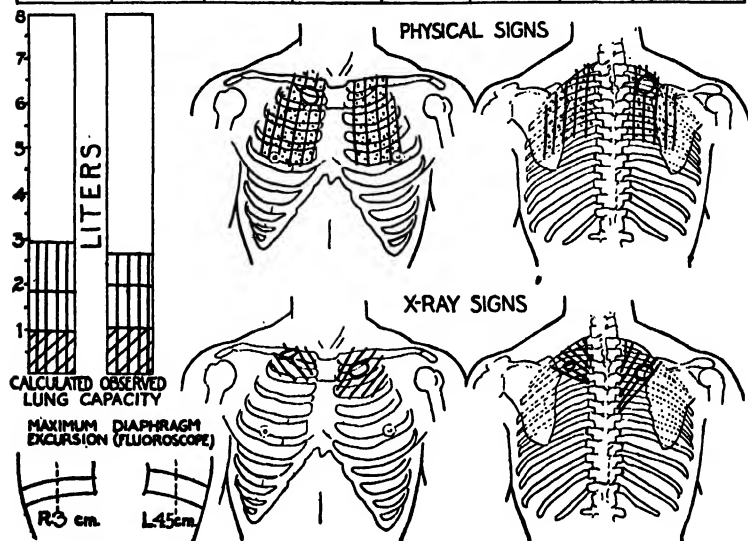
Height 172 cm.	Theoretical normal weight.....	kg. 68.0
Present weight.....	.....	67.5
Patient's idea of normal weight.....	.....	66.0
Date of highest weight 0 months ago.....	.....	67.5
“ “ lowest “ 6 “ “ .....	.....	59.0
Treatment duration 11 months.		

*Physical Signs.*—April 9, 1917. Right, slight impairment of resonance; slight increase in sharpness of breath sounds; no râles. Left, slight dullness; breath sounds slightly increased in intensity; no râles.

*X-Ray Signs.*—April 7, 1917. Right apex moderately densely infiltrated and spotted; rest of lung normal. Left apex moderately densely infiltrated and spotted; rest of lung normal. Mediastinal contents slightly to the left.

# **No. 9 (CASE 4061)**

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100% LUNG CAP. CHEST VOL.	RATIO 100% VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	*CHEST VOLUME*			
REST	cm. 15.5	cm. 15.8	cm. 20.8	liters 5.09	liters 1.95	38.2	32.4
MAX. INSP.	15.5	17.0	21.3	5.34	2.60	48.7	—
MAX. EXP.	15.5	15.2	20.3	4.78	1.0	20.9	—



TEXT-FIG. 9.

*No. 9 (Case 4061).*—Female, saleswoman; age 34 years. Advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 48 months ago with malaise, cough, expectoration, and slight loss of weight. Under sanatorium treatment general condition has slightly improved, the physical signs remaining the same.

Height 160 cm.	Theoretical normal weight.....	58.5
Present weight.....		45.5
Patient's idea of normal weight.....		50.0
Date of highest weight 6 years ago.....		50.0
“ “ lowest “ 12 months ago.....		40.5
Treatment duration 10 months.		

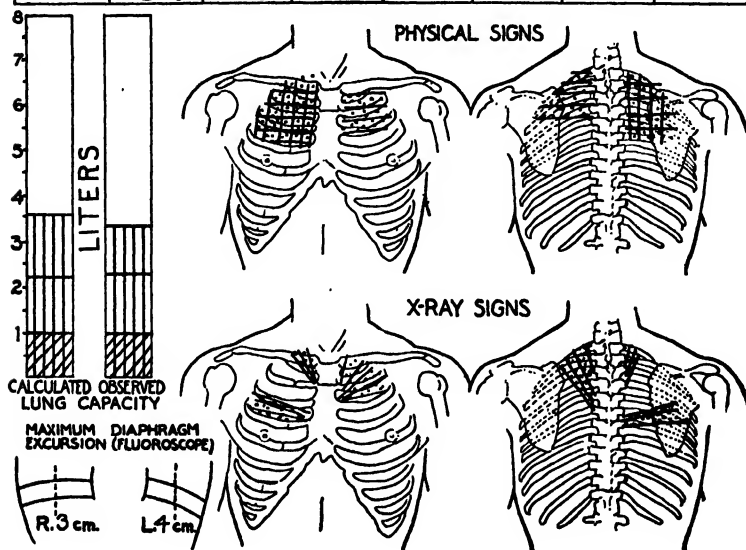
*Physical Signs.*—April 9, 1917. Right, slight dullness; breath sounds are moderately harsh; râles occur at apex to fifth rib anteriorly and to sixth spine posteriorly with aid of cough; cavernous breathing at right apex. Left, moderate dullness; breath sounds are markedly harsh; râles of medium moist type to fifth rib anteriorly and to sixth spine posteriorly, greatly increased by aid of cough.

*X-Ray Signs.*—April 7, 1917. Right apex and first interspace densely infiltrated; cavity under the clavicle  $1\frac{1}{2}$  by  $3\frac{1}{2}$  cm. Left apex and first and second interspaces quite densely infiltrated; cavity below the clavicle 4 by 3 cm. Mediastinal contents centrally placed.



# No. 10 CASE 4314

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTEPOST.	TRANSVERSE	*CHEST VOLUME*			
REST	cm. 18.0	cm. 14.6	cm. 22.1	liters 5.81	liters 2.25	38.8	40.5
MAX. INSP.	18.0	14.9	24.0	6.43	3.35	52.1	—
MAX. EXP.	18.0	13.9	21.5	5.38	1.0	18.6	—



TEXT-FIG. 10.

No. 10 (Case 4314).—Female, housewife; age 30 years. Moderately advanced; active. Sputum — —, on admission, in course of treatment, and at present.

Onset 16 months ago with malaise, cough, and expectoration; loss of strength followed; slight temperature. Under treatment symptoms became stationary, but lesion has progressed slightly.

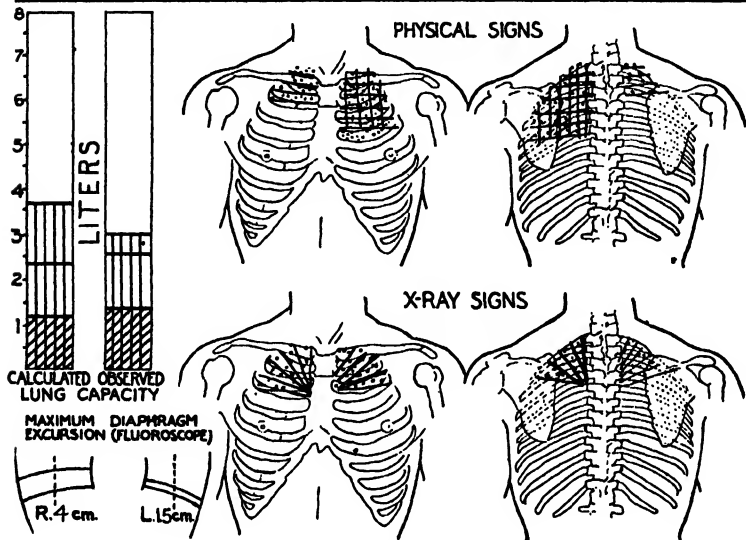
Height 155 cm.	Theoretical normal weight.....	kg. 53.5
Present weight.....		40.5
Patient's idea of normal weight.....		47.5
Date of highest weight 8 years ago.....		47.5
“ “ lowest “ 3 months ago.....		38.5
Treatment duration 3 months.		

**Physical Signs.**—April 9, 1917. Right, marked impairment of resonance; breath sounds harsh; coarse moist râles on breathing, with an increase in number with cough to fourth rib anteriorly and sixth spine posteriorly. Left, slight impairment of resonance; no change in breath sounds; fine moist râles increased by cough to third rib anteriorly and fourth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex densely spotted; first and second interspaces clear; third interspace moderately densely spotted. Left apex and first and second interspaces moderately densely spotted. Mediastinal contents normal.

# No. 11 (CASE 4059)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	18.3	15.9	21.7	6.32	2.5	39.6	27.0
MAX. INSP.	18.3	16.4	22.3	6.68	3.0	44.9	—
MAX. EXP.	18.3	14.9	21.0	5.73	1.3	23.6	—



TEXT-FIG. 11.

No. 11 (Case 4059).—Female, stenographer; age 20 years. Moderately advanced; active. Sputum — + +, on admission, in course of treatment, and at present.

Onset 18 months ago with cough, malaise, and loss of weight. Under sanatorium treatment general condition improved and symptoms diminished markedly although physical signs increased.

Height 160 cm.	Theoretical normal weight.....	54.5
Present weight.....		46.0
Patient's idea of normal weight.....		45.5
Date of highest weight 3 months ago.....		48.5
“ “ lowest “ 10 “ “.....		37.5

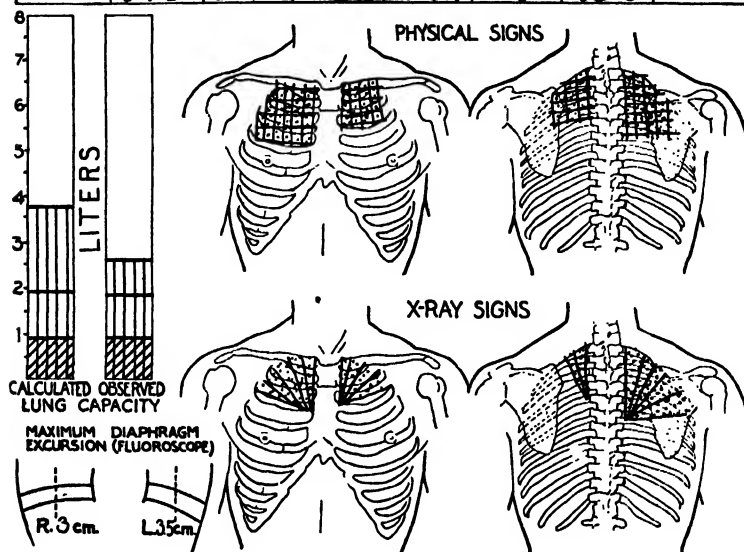
Treatment duration 10 months.

**Physical Signs.**—April 9, 1917. Right, slight dullness; breath sounds slightly harsh; fine moist râles to second rib anteriorly and to third spine posteriorly, much increased with cough. Upper part of left lung, marked dullness at apex to third rib anteriorly, and to sixth spine posteriorly; breath sounds amphoric anteriorly, harsh posteriorly; medium moist râles to fourth rib anteriorly and to fifth spine posteriorly. Cough increased the number of signs.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately spotted. Left apex and first interspace very dense; second interspace very finely stippled. Mediastinal contents entirely to the left. Right lung area much increased.

# No. 12 (CASE 3882)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	14.1	15.4	23.6	5.12	1.8	35.3	31.5
MAX. INSP.	14.1	16.4	25.1	6.80	2.6	38.2	—
MAX. EXP.	14.1	14.8	23.2	4.84	0.9	18.6	—



TEXT-FIG. 12.

No. 12 (Case 3882).—Female, clerk; age 21 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset with cough 3 years ago; malaise and loss of weight followed. Later a high temperature with right base fluid which cleared up spontaneously in 60 days. Under treatment, symptoms have markedly improved and lesion has become stationary.

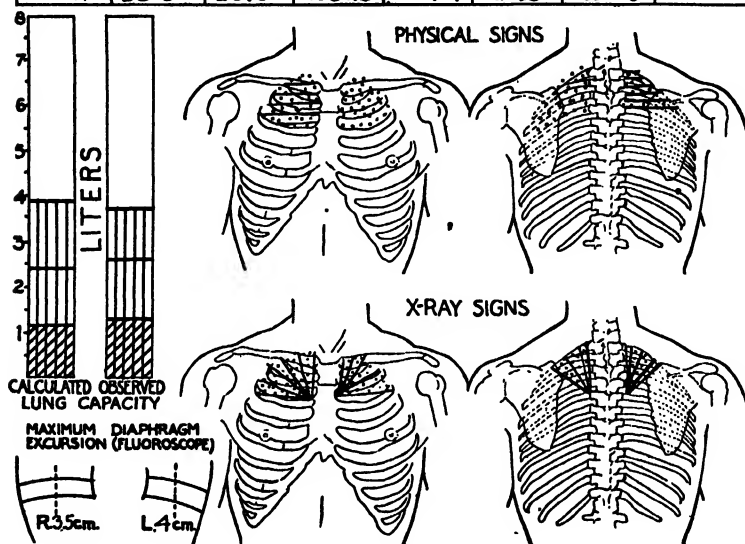
Height 157 cm.	Theoretical normal weight.....	53.5	kg.
Present weight.....		55.0	
Patient's idea of normal weight.....		53.5	
Date of highest weight 1913.....		56.0	
“ “ lowest “ 16 months ago.....		46.0	
Treatment duration 15 months.			

**Physical Signs.**—April 9, 1917. Right, marked dullness to fourth rib; harsh breathing; medium moist râles numerous with, and fewer without aid of cough to fourth rib anteriorly and sixth spine posteriorly. Left, marked dullness to third rib; harsh breathing; râles are rather moist, increased by cough, to third rib anteriorly and fourth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely stippled and striated. Left apex and first interspace moderately densely stippled and striated. Mediastinal contents normal.

## No. 13 (CASE 4191)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 16.5	cm. 16.4	cm. 23.9	liters 6.46	liters 2.6	40.3	38.6
MAX. INSP.	16.5	17.6	24.8	7.20	3.7	51.4	—
MAX. EXP.	16.5	15.0	23.2	5.74	1.2	20.9	—



TEXT-FIG. 13.

No. 13 (Case 4191).—Female, factory worker; age 18 years. Moderately advanced; active. Sputum — — +, on admission, in course of treatment, and at present.

Onset 6 months ago with hemoptysis. With sanatorium treatment symptoms markedly decreased, but physical examination shows an increase in the size of the lesion.

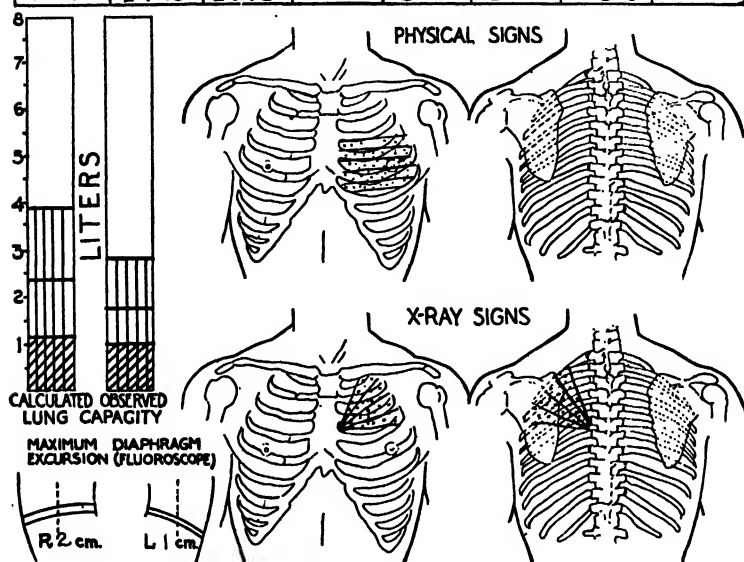
Height 157 cm.	Theoretical normal weight.....	kg. 51.0
Present weight.....		57.0
Patient's idea of normal weight.....		52.0
Date of highest weight 2 months ago.....		57.5
" " lowest " 11 " ".....		48.0
Treatment duration 6 months.		

**Physical Signs.**—April 9, 1917. Right, slightly dull; breath sounds slightly harsh; medium moist râles to third rib anteriorly and to fourth spine posteriorly, very much increased by cough. Left, no dullness; breath sounds slightly harsh; medium moist râles to third rib anteriorly and to fourth spine posteriorly, much increased with the aid of cough.

**X-Ray Signs.**—April 7, 1917. Right apex and first and second interspaces moderately densely infiltrated. Left apex and first and second interspaces moderately densely infiltrated. Mediastinal contents a little to the left.

## No. 14 (CASE 4044)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE-POST.	TRANSVERSE	*CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	17.2	16.4	23.5	6.62	1.70	25.6	27.2
MAX. INSP.	17.2	17.6	24.3	7.34	2.80	38.1	—
MAX. EXP.	17.2	15.8	23.0	6.25	1.0	16.0	—



TEXT-FIG. 14.

No. 14 (Case 4044).—Female, stenographer; age 17 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset 17 months ago with cough and expectoration; no physical discomfort. Under treatment cough has diminished and weight increased. Physical signs have diminished markedly.

Height 156 cm.	Theoretical normal weight.....	50.5
Present weight.....		59.0
Patient's idea of normal weight.....		48.5
Date of highest weight 1 month ago.....		60.0
“ “ lowest “ 19 months “ .....		47.5
Treatment duration 11 months.		

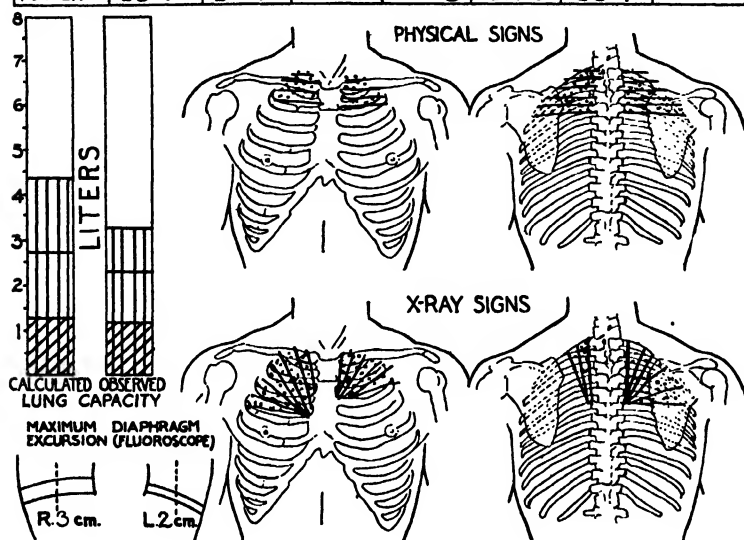
**Physical Signs.**—April 9, 1917. Right lung normal. Left, slight dullness over third, fourth, and fifth interspaces anteriorly; breathing harsh over this area; fine rales after cough in this area. Posteriorly there are no physical signs.

**X-Ray Signs.**—April 7, 1917. Right lung clear. Left, first, second, and third interspaces moderately densely infiltrated. Mediastinal contents markedly to the left. Right lung area is much increased.



## No. 16 (CASE 3947)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE-POST.	TRANSVERSE	CHEST VOLUME*			
	cm.	cm.	cm.	liters	liters		
REST	19.7	15.6	23.3	7.15	2.20	30.8	29.3
MAX. INSP.	19.7	16.4	24.8	8.00	3.20	40.0	—
MAX. EXP.	19.7	14.7	22.7	6.58	1.10	16.7	—



TEXT-FIG. 16.

No. 16 (Case 3947).—Female, cashier; age 20 years. Moderately advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 14 months ago with malaise, loss of weight, pleurisy, and cough with expectoration. Under sanatorium treatment general condition and weight improved, and physical signs have diminished.

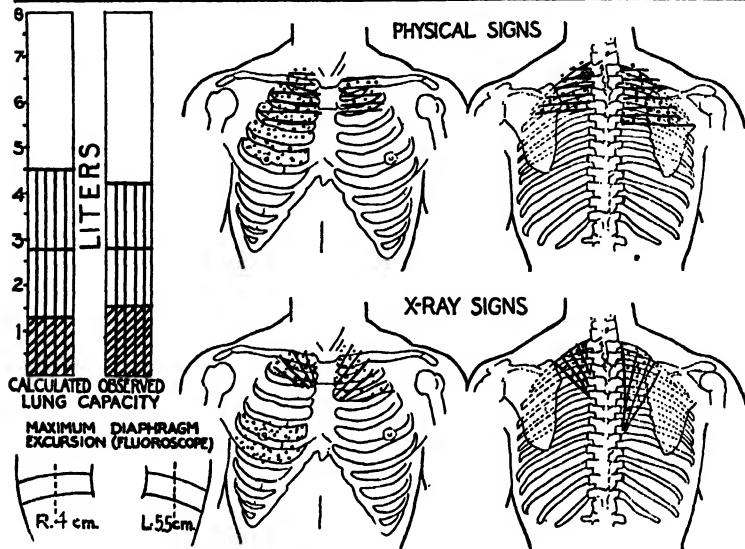
	kg.
Height 156 cm. Theoretical normal weight.....	52.0
Present weight.....	50.0
Patient's idea of normal weight.....	52.0
Date of highest weight 36 months ago.....	60.0
" " lowest " 14 " ".....	47.0
Treatment duration 12 months.	

**Physical Signs.**—April 9, 1917. Right, slight dullness to second rib; no breath sound change; râles after cough to second rib anteriorly and fourth spine posteriorly. Left lesion gives the same signs to almost exactly the same extent. In 12 months the physical signs have diminished about one-half.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace moderately densely spotted and striated; second and third interspaces finely stippled. Left apex and first and second interspaces present a mixture of coarse and fine stippings. Mediastinal contents normal.

# No. 17 (CASE 4283)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP CHEST VOL.
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
REST	cm. 18.9	cm. 16.3	cm. 24.6	liters 7.57	liters 2.8	37.0	34.4
MAX. INSP.	18.9	17.1	25.3	8.18	4.2	51.3	—
MAX. EXP.	18.9	15.6	23.3	6.86	1.6	23.4	—



TEXT-FIG. 17.

No. 17 (Case 4283).—Female, domestic; age 39 years. Moderately advanced; inactive. Sputum + + +, on admission, in course of treatment, and at present.

Onset insidious 6 months ago, with malaise, slight cough, and slight hemoptysis. Under sanatorium treatment symptoms disappeared and physical signs have diminished in number.

Height 171 cm.	Theoretical normal weight.....	67.5
Present weight.....		56.5
Patient's idea of normal weight.....		54.5
Date of highest weight 40 months ago.....		58.0
" " lowest " 9 " ".....		51.0
Treatment duration 3 months.		

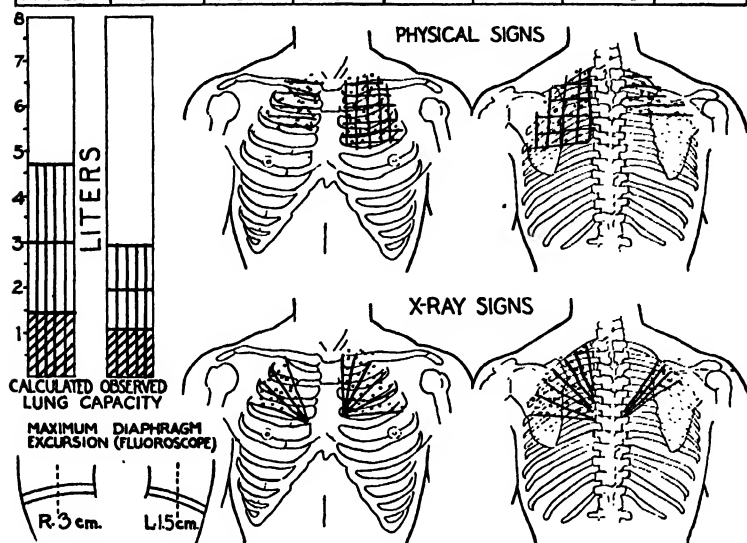
**Physical Signs.**—April 9, 1917. Right, resonance impaired to third rib; harsh breathing; fine rales after cough to fourth interspace anteriorly and fifth spine posteriorly. Left, resonance impaired to second rib; breathing is diminished in intensity; fine rales after cough to second rib anteriorly and fourth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex and first interspace slightly stippled and striated; the fourth and fifth interspaces are very slightly stippled. Left apex and first and second interspaces are slightly striated and stippled. Mediastinal contents centrally placed.



# No. 18 (CASE 4264)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANT. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	19.4	16.4	25.6	8.15	—	—	23.4
MAX. INSP.	19.4	17.3	26.0	8.72	2.9	33.2	—
MAX. EXP.	19.4	15.7	25.0	7.62	1.0	13.1	—



TEXT-FIG. 18.

No. 18 (Case 4264).—Female, factory worker; age 19 years. Moderately advanced; active. Sputum + — —, on admission, in course of treatment, and at present.

Onset 12 months ago with cough and expectoration; later marked temperature developed. Under treatment symptoms have improved, but physical signs have increased slightly.

Height 157 cm.	Theoretical normal weight.....	kg. 52.5
Present weight.....		62.0
Patient's idea of normal weight.....		54.5
Date of highest weight 3 months ago.....		63.5
" " lowest " 9 " " .....		51.5

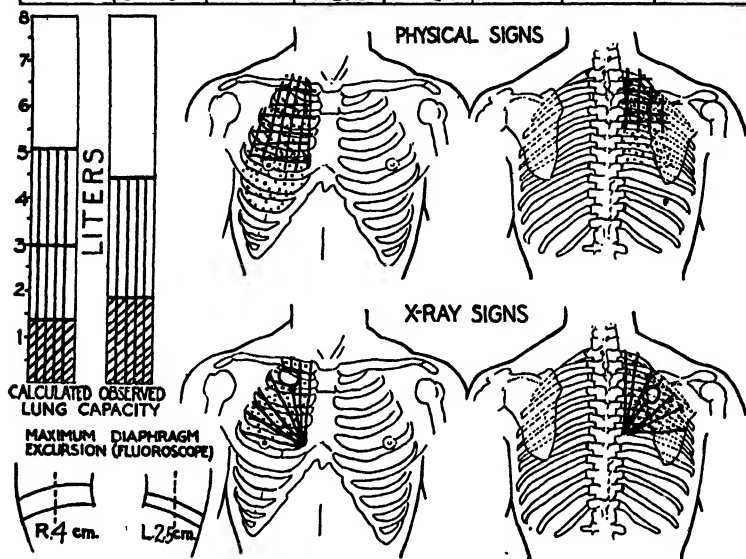
Treatment duration 5 months.

**Physical Signs.**—April 9, 1917. Right, slight impairment of resonance; breath sounds are a little increased in intensity; numerous fine and moist rales with cough to third rib anteriorly and fourth spine posteriorly; a few rales in this area on breathing without cough. Left, marked dullness; breath sounds are diminished in intensity; rales on breathing, but much increased in number by cough, extending to fourth rib anteriorly and sixth spine posteriorly.

**X-Ray Signs.**—April 7, 1917. Right apex clear; first, second, and third interspaces slightly spotted and striated. Left apex and first and second interspaces very dense; third interspace less dense. Mediastinal contents to the left. Right lung area increased.

No. 19 (CASE 3908)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY liters	RATIO 100 X LUNG CAP. CHEST VOL.	RATIO 100 X VITAL CAP. CHEST VOL.
	STERNUM	ANTE-POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters			
REST	18.5	17.3	25.3	8.11	—	—	30.1
MAX. INSP.	18.5	18.7	26.6	9.20	4.4	47.8	—
MAX. EXP.	18.5	16.2	24.8	7.43	1.9	24.5	—



TEXT-FIG. 19.

No. 19 (Case 3908).—Female, factory inspector; age 23 years. Advanced; active. Sputum + + +, on admission, in course of treatment, and at present.

Onset 25 months ago with malaise, slight loss of weight, slight cough, and slight temperature. Aphonia for last 10 months (tuberculous laryngitis). Under sanatorium rest temperature has become normal; lung lesion has progressed slightly.

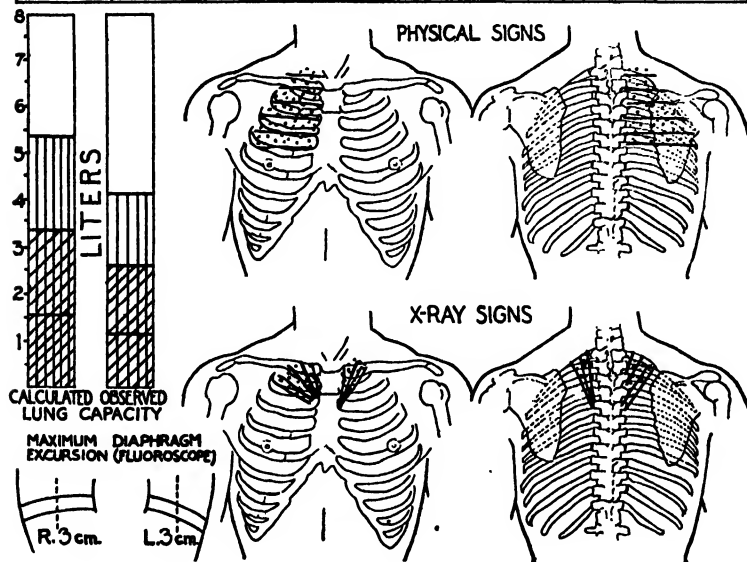
Height 173 cm.	Theoretical normal weight.....	64.0
Present weight.....		70.0
Patient's idea of normal weight.....		60.0
Date of highest weight 14 months ago.....		77.5
" " lowest " 2 " ".....		69.5
Treatment duration 14 months.		

**Physical Signs.**—April 9, 1917. Right, dull to fifth interspace; harsh breathing; numerous very moist rales without cough through the lung anteriorly, and to the seventh spine posteriorly. Left lung seems normal.

**X-Ray Signs.**—April 7, 1917. Right lung moderately densely spotted and striated to the fourth interspace; cavity under the clavicle 5 cm. in diameter. Left lung clear. Mediastinal contents very markedly to the right. Left lung area greatly increased.

## No. 20 (CASE 4103)

POSITION	CHEST DIMENSIONS				LUNG CAPACITY	RATIO 100 X LUNG CAP CHEST VOL	RATIO 100 X VITAL CAP CHEST VOL
	STERNUM	ANTE. POST.	TRANSVERSE	"CHEST VOLUME"			
	cm.	cm.	cm.	liters	liters		
REST	18.6	17.8	27.3	9.05	2.60	28.7	31.5
MAX. INSP.	18.6	18.5	28.5	9.80	4.15	42.3	—
MAX. EXP.	18.6	16.6	26.2	8.08	1.20	14.9	—



TEXT-FIG. 20.

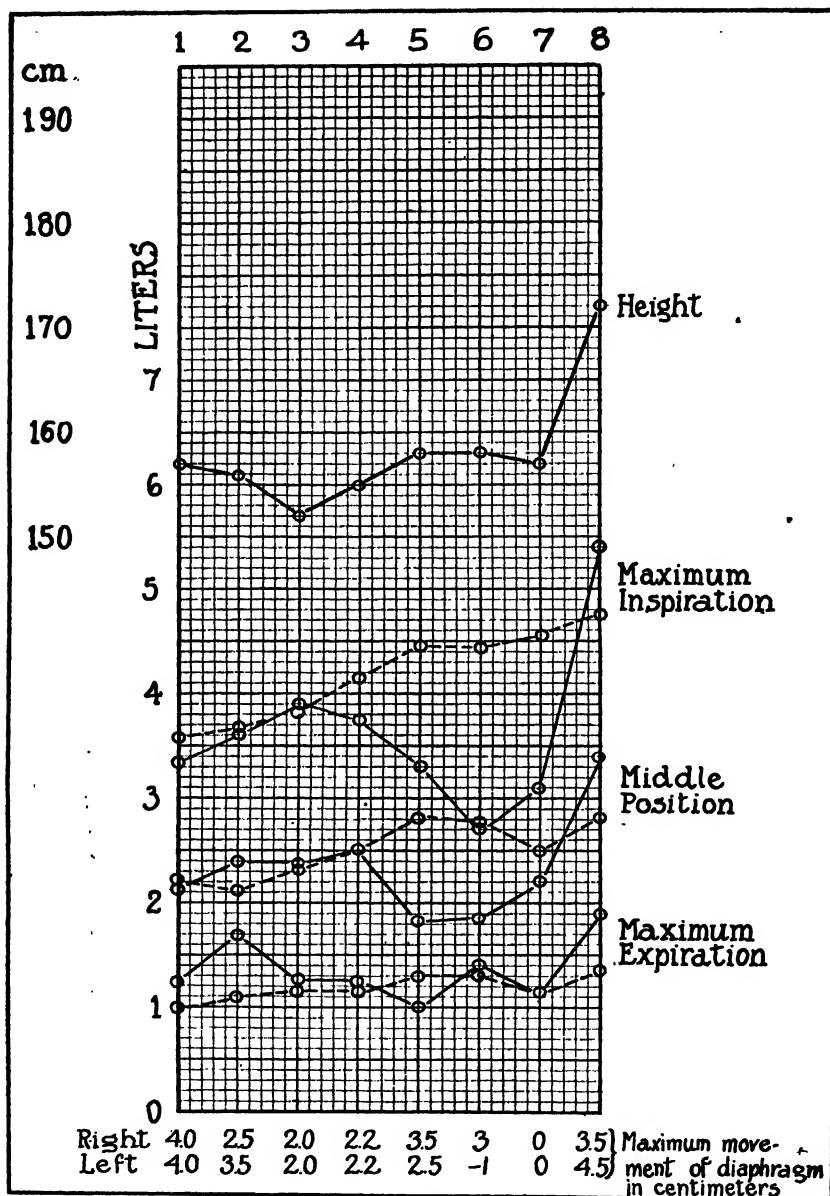
No. 20 (Case 4103).—Female, cotton mill worker; age 25 years. Moderately advanced; active. Sputum + + -, on admission, in course of treatment, and at present.

Onset 14 months ago with malaise, loss of weight, cough, and hemoptysis. Under sanatorium treatment the symptoms almost disappeared, and the signs are greatly diminished in number.

Height	170 cm.	Theoretical normal weight.....	kg. 62.5
Present weight.....			76.0
Patient's idea of normal weight.....			68.0
Date of highest weight	3 months ago.....		79.0
" " lowest	" 15 " ".....		61.0
Treatment duration 9 months.			

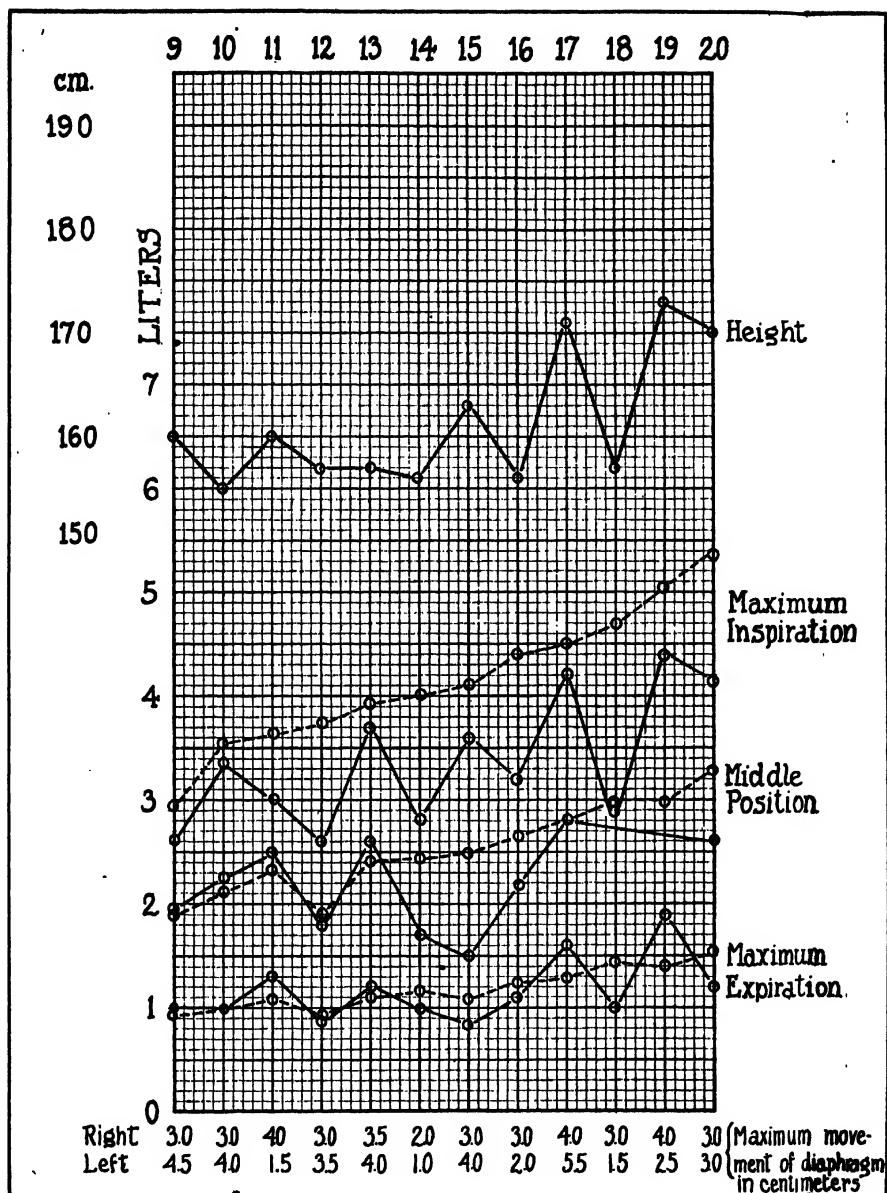
*Physical Signs.*—April 9, 1917. Right, moderate impairment of resonance; breath sounds increased in intensity and medium moist râles from apex to fourth rib anteriorly and to sixth spine posteriorly with aid of cough. Left lung seems normal.

*X-Ray Signs.*—April 7, 1917. Right apex and first interspace moderately densely infiltrated. Left apex slightly infiltrated. Mediastinal contents normal.



The numbers below indicate the maximum excursion of the right and left diaphragm. The numbers above the chart refer to the individual diagrams and descriptions.

TEXT-FIG. 21. Lung volumes in women with incipient pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic-measurements.



TEXT-FIG. 22. Lung volumes in women with moderately advanced and advanced (Nos. 9 and 19) pulmonary tuberculosis as determined (solid lines) and calculated (broken lines) from thoracic measurements.



## THE COLORIMETRIC DETERMINATION OF HEMOGLOBIN.\*

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The method to be described for the determination of hemoglobin depends upon the comparison, in a colorimeter, of carbon monoxide hemoglobin solutions, one of which has a known hemoglobin content. Hoppe-Seyler (1) was the first to describe carbon monoxide hemoglobin and to make use of this stable combination for estimating the hemoglobin content of blood. He devised a "double pipette" for comparing the unknown carbon monoxide hemoglobin solution with the standard carbon monoxide hemoglobin solution, prepared from hemoglobin crystals; but the method never came into general use, because of the many technical difficulties involved. Haldane (2) suggested a much simpler method for comparing carbon monoxide solutions, using the apparatus employed by Gowers (3) for comparing oxyhemoglobin solutions with a picro-carmin standard. This apparatus was later employed by Sahli (4) who prepared an acid hematin standard by adding dilute hydrochloric acid to blood.

A critical discussion of the various methods in use for the estimating of hemoglobin is beyond the scope of this paper. As Haldane (2) has pointed out, artificially colored solutions and tinted glass present great difficulties in standardization with a definite strength of hemoglobin solution. With a certain strength of color solution or tinted glass, it is possible to imitate quite perfectly the tint of a given hemoglobin solution provided the quality of light remains the same. Any variation from these standard conditions, either in quality of light or strength of hemoglobin in solution leads to serious errors. Haldane attempted to overcome the objection by preparing a carbon

A brief report of this paper is given in *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 175.

monoxide hemoglobin standard (1 per cent solution of a blood having an oxygen capacity of 18.5 per cent) which he considered permanent when kept sealed in a small test-tube in an atmosphere of carbon monoxide. 0.020 cc. of blood, placed in a similar sized and shaped graduated (on scale of 100) test-tube with a small amount of water saturated with carbon monoxide by means of ordinary illuminating gas, are diluted with water, drop by drop, until the unknown and standard tubes match in color, whence the percentage of hemoglobin in the unknown may be read off on the graduated scale. In principle, Haldane's method is sound; but certain practical difficulties arise. The standard is not so permanent as was at first thought, and, when water is used, it has been shown by Krogh (5) that the full color of the solution is slow in reaching its maximum. The further criticism is that the method is time-consuming and cumbersome—adding water, drop by drop, and shaking after each addition. Also the color comparison in the two tubes is not sharp. This method has one distinct advantage in that it may be used for the determination of hemoglobin in any species of animal.

Sahli employed Haldane's apparatus and technique; except that he used, as a standard, blood to which dilute hydrochloric acid had been added. There are three serious objections to Sahli's method: first, the standard is not permanent; second, there is considerable delay in the development of the maximum or permanent color, amounting, according to Meyer and Butterfield (6), in some instances to 20 per cent; and third, it cannot be used for the blood of different species.

The spectrophotometer has undoubted accuracy in the hands of skilled operators; but the expense and unavailability together with the difficult technique involved, make it impracticable for general use.

The great variety of methods and apparatus which have been proposed offer eloquent testimony to the unsatisfactory means for the determination of hemoglobin. There is great need for a rapid, accurate, and universally standard method for the estimation of hemoglobin in experimental work and the study of blood diseases in the clinics.

The method which we have found to fulfil the above conditions is as follows:



*Method.*

Blood is obtained in the usual manner by pricking the finger or lobe of the ear. A 1 per cent solution of blood is made by drawing 0.05 cc. into a special pipette and transferring into 5 cc. of 0.4 per cent ammonia solution—accurately measured with a calibrated pipette or burette into a  $12 \times 120$  mm. test-tube. The blood pipette is rinsed out by drawing into it two or three times the ammonia solution. Ordinary illuminating gas is bubbled rapidly through the ammonia blood solution for 30 seconds, after which it is compared in a Duboscq colorimeter with a standard carbon monoxide hemoglobin solution set at 10. The average of at least four readings is taken. The calculation is simple,  $\frac{10}{R} \times 100 =$  per cent hemoglobin.

*Manner of Obtaining Blood.*—With sufficient care the usual clinical method for obtaining small amounts of blood by pricking the ear or finger is satisfactory. A free flow is essential. Any undue manipulation or squeezing of the part should be avoided because an error of 5 or 10 per cent may be introduced by diluting the blood with tissue juice. Where there is marked anemia requiring larger amounts of blood than 0.05 cc., or where there is difficulty in obtaining blood from the ear or finger, venous puncture should be used, coagulation being prevented with oxalate or citrate salts. It is often practical and convenient to combine the determinations of hemoglobin with other blood analyses, where venous puncture is required. If blood has been drawn by venous puncture care must be taken that the corpuscles and serum are well mixed before filling the pipette. The blood should never be shaken violently before measuring, because it becomes filled with air bubbles. The mixture of corpuscles and serum may best be accomplished by first giving the receptacle a circular motion and finally stirring briskly with a glass rod or measuring pipette which is filled while stirring the blood.

*Pipette for Measuring Blood.*—The pipettes are made of millimeter glass tubing calibrated to contain 0.05 cc. and 0.10 cc. The pipettes are easily made in any laboratory from straight tubing, and require no blowing, the point being rounded off on an emery wheel. In this way time and expense are saved, since pipettes

obtained from glass blowers require recalibration before use. It has been found that water may be used for this calibration, as pipettes which have been calibrated with both mercury and water check sufficiently well. The advantage of having the pipette calibrated to contain 0.10 cc. as well as 0.05 cc. is obvious. In bloods with a low hemoglobin content, 0.05 cc. may not be sufficient to give the color necessary for accurate color comparison in the colorimeter. A pipette of this type, and used in the manner described, is capable of measuring 0.05 cc. of blood with an accuracy of 0.2 per cent.

*Ammonia Solution.*—Ammonia solutions, containing 4 cc. of strong ammonia in 1 liter of water, suggested by Krogh (5) are used, because the full color of the carbon monoxide hemoglobin develops at once.

*Saturation with Carbon Monoxide.*—Ordinary illuminating gas as a source of carbon monoxide has proven entirely satisfactory. It was thought that there might be substances other than carbon monoxide in the gas which might form hemoglobin compounds and interfere with the determination. Accordingly, pure carbon monoxide was prepared by heating oxalic acid with concentrated sulfuric acid and passing the gas produced through sodium hydroxide to free it from carbon dioxide. As far as could be determined on comparison of the two solutions in the colorimeter the colors were identical. Oxyhemoglobin solutions are very unstable. Hence it is necessary after transferring the blood to the ammonia solution, to saturate with carbon monoxide within an hour. After saturation with carbon monoxide, the solution may, on carefully stoppering and protecting from light, be placed in the ice box and the determination made at leisure. Saturation of the blood should be carried out under a hood. If the laboratory does not possess a hood, the saturation may be accomplished under a funnel, attached to a small water vacuum pump, to remove the gas.

*Standard Hemoglobin Solution.*—Haldane's standard of a 1 per cent solution of a blood having an oxygen capacity of 18.5 per cent is used. It has been shown by Haldane and Smith (7), Butterfield (8), Barcroft (9), and others, that the oxygen capacity of the blood depends upon its hemoglobin content. A blood of 18.5 per cent

oxygen capacity contains approximately 14 gm. hemoglobin per 100 cc. Although the blood of normal men in this country, as shown by Meyer and Butterfield (6), Williamson (10), and also as we have found by use of the method here described, contains on the average 16.6 gm. of hemoglobin, which would correspond to an oxygen capacity of about 22 per cent, it was thought best to keep Haldane's standard for the present. It is a simple matter to compute the gm. of hemoglobin in any given blood from the results obtained.

The standard hemoglobin solution is prepared as follows: A quantity of defibrinated human or ox blood is obtained. The oxygen capacity is determined by the method of Van Slyke (11). The blood may also be standardized by a spectrophotometer or solutions made from hemoglobin crystals prepared in the manner described by Butterfield (8). We have checked several times our standard and found that the oxygen capacity method for standardization is most convenient and satisfactory. The blood is diluted with 0.4 per cent ammonia solution so as to make a 20 per cent solution of a blood with an oxygen capacity of 18.5 per cent. This 20 per cent blood solution is then saturated with carbon monoxide by bubbling through it illuminating gas for 10 minutes. A drop of caprylic alcohol prevents troublesome foaming. The glass tube through which the gas is passed into the blood solution is withdrawn slowly and the bottle stoppered immediately. Rubber corks must not be used in connection with hemoglobin solutions. The cork should be sealed in with paraffin and the solution, protected from light, kept in the ice chest. Such a solution thus protected will keep for months. Several solutions now nearly a year old prepared in this manner have shown no deterioration. 5 cc. of this 20 per cent blood solution made up to 100 cc. with 0.4 cc. of ammonia solution and saturated with carbon monoxide, make the 1 per cent standard for use in the colorimeter and may be prepared from time to time as desired. The 1 per cent standard for routine use may be kept in a dark glass or black painted aspirator bottle, the lower opening of which is provided with a cork, through which passes a glass tube with a ground glass cock for withdrawing small amounts of solution. A glass tube is put through the cork in the top of the bottle and extends to the bottom. Both corks should be sealed with paraffin. This glass tube is con-

nected with an open gas fixture in order that when solution is withdrawn from the bottom, gas rather than air will enter to replace it. Solutions thus prepared may keep for several weeks; but, as a precaution, it is advisable to make fresh 1 per cent solutions frequently; *i.e.*, every 2 or 3 weeks. It should be remembered that dilute hemoglobin solutions are less stable than concentrated solutions; and that hemoglobin solutions keep best in the cold and protected from light. The first indication of solution deterioration is a change in color from the characteristic cherry-red of carbon monoxide hemoglobin to a red with a brownish tinge, due to the formation of methemoglobin.

*Comparison in Colorimeters.*—The Duboscq or Kober colorimeters have proven to be by far the most accurate and satisfactory instruments for this colorimetric work. Other colorimeters, however, may be used. The difficulties encountered are those inherent in all colorimetric work and in this connection reference to Kober's (12) article may be made. The color of the carbon monoxide hemoglobin, because of the relatively low stimulus threshold for the eye, is admirably suited to colorimeter comparison, slight differences being easily detected. We prefer to use the daylight from a north window. Satisfactory results are, however, obtainable with artificial light when "daylight glass" is used between the source of light and the solution. Considerable experimentation with light filters has failed to improve on the accuracy with which the comparison may be made. No difficulty should be experienced in making the readings check within 0.2 of a single division on the colorimeter scale.

Color comparisons are most accurate when the unknown hemoglobin solution reading falls between 9 and 11 on the colorimeter scale. If the reading of the unknown falls below 8 or above 12, another sample should be taken and the dilution made such that the reading will fall within these limits. This is easily accomplished by varying the amounts of blood and ammonia solution, making the necessary correction in the colorimeter. 2 cc. of solution is adequate for the Kober instrument and 5 cc. for the Duboscq. If a Duboscq or a Kober colorimeter is not available, the Hellige instrument may be used. The 1 per cent hemoglobin standard may be sealed with paraffin into the wedge and the wedge, when not in use, kept in the ice

box and protected from light. Attention should be called to the fact that the scales of the Hellige colorimeter are often inaccurately placed. The standard solution must be checked against itself and the scale adjusted so as to read 100 when the color in the cup and wedge match. We have found more difficulty in obtaining accurate checks with this instrument than with the Duboscq or Kober colorimeters; but with care the error should not exceed 2 per cent. 2 cc. of solution is sufficient for the Hellige cup, hence the Sahli pipette, which contains 0.020 cc. of blood, may be used with this colorimeter. As large errors have been found in the calibration of the Sahli pipettes, it is necessary to recalibrate them before using.

*Accuracy of the Method.*—The accuracy of the method depends to a large extent on the care of the operator in carrying out the various details of the technique. The several steps involved in the method are common chemical procedures with known limits of accuracy. With care duplicate determinations are close and the error should not be more than 1 per cent. A series of ten determinations was made on the same blood. In eight of the ten, the first reading of the colorimeter was in every case exactly the same. In the two remaining a difference of 0.1 of a division on the colorimeter scale occurred.

In the following table are presented a few determinations by the method described using the Duboscq and Hellige colorimeters and comparing with the values estimated from the oxygen capacity

Blood sample.	Hemoglobin determinations.			Difference between Duboscq and oxygen capacity.	Percentage difference.
	Duboscq.	Hellige.	Oxygen capacity.		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
1	105.5	104.8	106.5	+1.0	0.94
2	95.2	92.0	94.2	-1.0	1.06
3	128.3	125.0	129.5	+1.2	0.93
4	112.5	115.2	111.7	-0.8	0.68
5	116.2	116.3	116.4	+0.2	0.17
6	79.4	84.0	80.5	+1.1	1.36
7	119.2	115.0	119.4	+0.2	0.17
8	84.0	85.0	83.2	-0.8	0.95
9	82.0	82.0	82.6	+0.6	0.73
10	108.6	—	108.1	-0.5	0.46

of the blood. The first ten blood samples of a large series were chosen and illustrate the error in estimation which may be expected. Except in blood Sample 6, no variation between the colorimetric determination (Duboscq) and the oxygen capacity method is greater than 1.0 per cent.

*Advantages.*—(1) Single determinations may be carried through in 2 minutes. (2) An accuracy within 1 per cent is easily obtained. (3) The standard solution is easily and adequately controlled. (4) Similar solutions are used for comparison, making the color fields, within the limits of the colorimeter, identical. (5) The apparatus required is found in any well equipped laboratory.

#### SUMMARY.

A method for the determination of hemoglobin colorimetrically with an accuracy of 1 per cent is described.

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## GASOMETRIC DETERMINATION OF THE OXYGEN AND HEMOGLOBIN OF BLOOD.

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The apparatus described in a previous paper<sup>1</sup> for determining the carbonic acid content of plasma may be used with equal facility for determining the oxygen content and the oxygen-binding capacity (hemoglobin) of blood.

For the determination of the *oxygen capacity* as a measure of the hemoglobin 3 or more cc. of blood are introduced into a separatory funnel or bottle and distributed in a thin layer about the inner wall, so that maximum contact with the air and complete saturation of the hemoglobin with oxygen are assured. The vessel is rotated for a few minutes so that the blood is kept in a thin layer, or it may be shaken for 15 minutes or longer on a mechanical shaker. The saturated blood is transferred to a heavy test-tube or cylinder.

The blood gas apparatus is now prepared by introducing into it five drops of redistilled caprylic alcohol and 6 cc. of ammonia solution made by diluting 4 cc. of concentrated ammonia to a liter. If saponin powder is available, as much is added to the 6 cc. of ammonia while in the cup of the apparatus as will stick to the end of a glass rod. After the ammonia has been introduced into the 50 cc. chamber of the apparatus the latter is evacuated in the manner described in the previous article, and the air is extracted from the ammonia solution by shaking for about 15 seconds. The extracted air is expelled, and the extraction completed to make sure that no air is left in the solution. Finally, about 2 cc. of the air-free ammonia are forced up into the cup of the apparatus.

The aerated blood is now thoroughly stirred with a rod to assure even distribution of the corpuscles, and a sample is drawn into a

<sup>1</sup> Van Slyke, D. D., *J. Biol. Chem.*, 1917, xxx, 347.

2 cc. pipette and run under the ammonia in the cup of the apparatus. (The lower delivery mark of the pipette should be 3 or 4 cm. above the tip. A pipette calibrated for complete delivery would be inconvenient for placing the entire sample of blood below the layer of ammonia.) The blood is now run from the cup into the 50 cc. chamber, the ammonia layer following the blood and washing it in. A few additional drops of ammonia may if necessary be added from a dropper to make the washing complete.

The blood and ammonia in the chamber are mixed and allowed to stand until the blood is *completely laked*. This requires about 30 seconds when saponin is present, 5 minutes when it is not. After laking is complete 0.4 cc. of a saturated potassium ferricyanide solution is introduced to set free the oxygen combined with the hemoglobin. (The cyanide solution is made air-free by boiling or by shaking in an evacuated flask and is kept in a burette under a layer of paraffin oil 2 or 3 cm. thick to exclude air.) The apparatus is now evacuated by lowering the levelling bulb until only a few drops of mercury remain above the lower stop-cock, and is shaken, preferably with a rotary motion, to whirl the blood in a thin layer around the wall of the chamber. If the blood was completely laked before the cyanide was added, extraction of the oxygen may be completed by half a minute of efficient shaking. The extracted solution may be drawn into the bulb of the apparatus below the lower cock and the extracted gas measured over mercury as in the determination of carbon dioxide.<sup>2</sup> Or, since the water does not absorb oxygen rapidly enough to cause an error, the solution may be left in the 50 cc. chamber during the reading of the gas volume, the levelling bulb being held at a sufficient height to balance the column of water solution.<sup>2</sup> Finally, in order to make certain that all the oxygen was obtained by the first extraction, the apparatus is evacuated once more and the blood shaken again for a half minute. If the reading shows no increase, it is evident that all this oxygen was extracted by the first evacuation. If there is an increase the extraction must be repeated again. If the blood is completely laked before addition of the ferricyanide, the first shaking practically always removes all the oxygen

<sup>2</sup> Van Slyke, *J. Biol. Chem.*, 1917, xxx, 353.



from the blood solution. Even when laking has been incomplete, however, or the first extraction of the blood otherwise made incomplete, the determination is not lost; for the right result will be obtained if the extraction is repeated until the reading becomes constant.

After each analysis it is well to wash out the 50 cc. chamber of the apparatus with the dilute ammonia solution, as a black precipitate is formed by reaction of the reagents with the mercury. Unless this precipitate is removed it tends to coagulate after a few analyses and interfere with further determinations.

After the blood has been saturated with air the entire procedure above outlined, including the final cleaning of the apparatus, is done in routine determinations in 7 or 8 minutes.

In order to calculate the oxygen bound by the hemoglobin it is necessary to subtract from the gas measured the volume of air physically dissolved by the 2 cc. of blood at atmospheric pressure and the prevailing room temperature. The volume of gas thus corrected may be reduced to standard conditions by multiplying

by  $(0.999 - 0.0046 t) \times \frac{\text{barometer}}{760}$ ,  $t$  being the temperature in de-

grees centigrade. The volume of air dissolved by the blood at different temperatures are given in Table I. They are calculated in accordance with Bohr's finding, that the solubility of gases in average whole blood is 90 per cent of their solubility in water. The table also gives factors by which one may transpose the readings directly into terms of volume per cent of chemically bound oxygen in the blood, or of per cent hemoglobin on the basis of Haldane's normal average, 18.5 per cent of oxygen in the blood being taken as equivalent to 100 per cent hemoglobin.<sup>3</sup>

Unless one is well experienced with the conditions used for saturating the blood with oxygen, it is advisable, after one portion of a blood sample has been analyzed, to aerate the remainder a second time and repeat the determination, in order to make certain that the hemoglobin of the first portion was completely saturated with oxygen.

<sup>3</sup> Haldane, J., and Smith, J. L., *J. Physiol.*, 1899-1900, xxv, 331.

TABLE I.

*Factors for Calculating Results from Analysis of 2 Cc. of Blood Saturated with Air.*

Temperature.	Air physically, dissolved by 2 cc. of blood. Subtract from gas volume read in order to obtain corrected gas volume, representing O <sub>2</sub> set free from hemoglobin.	Factor by which corrected gas volume is multiplied in order to give:	
		Oxygen chemically bound by 100 cc. of blood.	Per cent hemoglobin, calculated on the basis of 18.5 per cent oxygen = 100 per cent hemoglobin.
°C.	cc.	cc.	per cent
15	0.037	$46.5 \times \frac{B}{760}$	$251 \times \frac{B}{760}$
16	0.036	46.3 "	250 "
17	0.036	46.0 "	249 "
18	0.035	45.8 "	247 "
19	0.035	45.6 "	246 "
20	0.034	45.4 "	245 "
21	0.033	45.1 "	244 "
22	0.033	44.9 "	242 "
23	0.032	44.7 "	241 "
24	0.032	44.4 "	240 "
25	0.031	44.2 "	239 "
26	0.030	44.0 "	237 "
27	0.030	43.7 "	236 "
28	0.029	43.5 "	235 "
29	0.029	43.3 "	234 "
30	0.028	43.1 "	233 "

The following example illustrates the calculation:

Observed gas volume, at 20°, 750 mm..... 0.450 cc.

Correction for dissolved air..... 0.034 "

Corrected gas volume..... 0.416 "

$0.416 \times 44.8 = 18.65$  volume per cent oxygen.

$0.416 \times 243 = 101$  per cent hemoglobin.

While the above description and the table are prepared to fit the analysis of 2 cc. samples of blood, which seems the desirable amount for ordinary purposes, either more or less may be taken, the volumes of dilute ammonia and ferricyanide used being changed proportionally. The following data show that good results are obtained with as much as 3 or as little as 1 cc. of blood, although the error must be greater in the latter case because of the small volumes of gas obtained for the final reading.

TABLE II.

*Determinations of the Oxygen Capacity of Ox Blood, Using 1 to 3 Cc. Samples.*

Volume of blood sample.	Temperature.	Barometer.	Gas volume obtained.	Gas volume corrected for dissolved air.	Volume of O <sub>2</sub> reduced to 0°, 760 mm. bound by 100 cc. of blood.	Per cent hemoglobin, calculated on basis of 18.5 per cent O <sub>2</sub> = 100 per cent hemoglobin.
cc.	°C.	mm.	cc.	cc.	cc.	per cent
3.00	20	752	0.835	0.784	23.45	126.7
2.00	20	752	0.558	0.524	23.49	126.9
1.00	20	752	0.281	0.264	23.67	127.9

For the determination of the *oxygen content* of the venous blood as drawn from the body, the preliminary saturation with air is omitted, and precautions, such as are described by Lundsgaard in the accompanying paper,<sup>4</sup> are observed to prevent contact of the sample with air. From the point where the blood sample is transferred to the cup of the apparatus for analysis the technique is identical with that described above.

The calculation of the oxygen content is somewhat different from that of the oxygen capacity. The physically dissolved oxygen of venous blood is negligible, and the nitrogen as determined by Bohr is 0.9 volume per cent, reduced to 0°, 760 mm.<sup>5</sup> Therefore the volume of gas obtained in the apparatus is at once multiplied by the factor  $(0.999 - 0.0046 t) \times \frac{\text{barometer}}{760}$  to reduce it to standard conditions. In case 2 cc. of blood have been used, the values of this factor in Column 3 of Table I may be used; in this case they give the content of the blood in chemically bound oxygen plus the dissolved nitrogen gas. The result in cc. of gas per 100 cc. of blood is diminished by 0.9 cc. in order to correct for that amount of nitrogen gas present.

The nature of the results obtained with the method and the degree of accuracy indicated by the agreement of duplicates are shown by Lundsgaard's figures,<sup>4</sup> while Palmer's show the agreement of the gasometric with the colorimetric determination of hemoglobin<sup>6</sup> by

<sup>4</sup> Lundsgaard, C., *J. Biol. Chem.*, 1918, xxxiii, 133.

<sup>5</sup> Bohr, C., in Nagel, W., *Handb. Physiol. Menschen*, 1909, i, 117.

<sup>6</sup> Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119.

the most accurate use of the colorimetric technique. It consequently appears unnecessary to reproduce in this paper further data to illustrate the results obtained with the method.

#### SUMMARY.

The apparatus previously described for determination of carbon dioxide in blood is used with a similar technique for determination of oxygen. The oxygen is set free from combination with hemoglobin within the apparatus by addition of ferricyanide, is extracted in a Toricellian vacuum, and measured at atmospheric pressure, a few minutes sufficing for an accurate determination.

## STUDIES OF OXYGEN IN THE VENOUS BLOOD.

### I. TECHNIQUE AND RESULTS ON NORMAL INDIVIDUALS.

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The term oxygen content of the venous blood has been used in the literature in two senses. It is applied to the amount of oxygen in the blood in the right heart and also the amount in the venous blood of some organ or group of organs.

In the blood of the right side of the heart the oxygen can be determined by using the lungs plus a bag (1-5) or a part of the lungs (6) as a tonometer. The air in the lungs is brought to equilibrium with the blood gas and a sample of the air is analyzed. In animals, samples of blood can be drawn through a cannula introduced into the heart.

Samples of venous blood from single organs can be obtained in animals when the technical difficulties can be overcome. Extensive studies have been made in the last few years, particularly by Barcroft and his associates (7).<sup>1</sup>

In adults the superficial veins of the limbs and neck, particularly of the arm (vena mediana) are the only sources from which venous blood can be obtained.<sup>2</sup> That means that only blood coming from a limited region, consisting chiefly of muscles, can be studied. Consequently the field of investigation is limited. The conditions can be varied voluntarily only to a small extent. The results have to be interpreted chiefly in an empirical way and by means of general clinical observation. In spite, however, of limitations and *a priori*

<sup>1</sup> For bibliography see Barcroft (7).

<sup>2</sup> In the Pediatric Department of Johns Hopkins Hospital venous blood is obtained by puncturing the sinus sagittalis in babies. This method makes it possible to obtain venous blood without stasis from children where the superficial veins are too small.

theoretical difficulties in interpreting the results, the question of the amount of oxygen in the venous blood in health and disease has always been considered important by physiologists and clinicians, although few investigations have been made. Not only are the observations on pathological cases few, but the determinations on normal individuals are even more scarce. The reason for this is probably that we did not have any means of determining blood gases in small quantities of blood until the Haldane-Barcroft method was devised.

Kraus (8) determined the oxygen and  $\text{CO}_2$  in the venous blood of a series of normal and pathological cases. Using the old blood-gas pump it was necessary for him to draw large quantities of blood, a circumstance which prevented repeated determinations in the same person. He found in some instances of uncompensated heart cases very low values for the venous oxygen compared with that of normal individuals. Using the Haldane-Barcroft method, Morawitz and Röhmer (9) did eighteen determinations on three normal people, twenty determinations on nineteen patients with anemia, and one determination on a patient with polycythemia. In some instances they found an increased consumption,<sup>3</sup> in other instances a normal consumption in their patients. Means and Newburgh (10) using the Haldane-Barcroft method found, like Kraus, low values in four patients with uncompensated heart diseases compared with those found in three normal subjects.

We know that in normal individuals the oxygen content of the venous blood is about 13.5 volume per cent, or two-thirds of the total amount in the blood when it is saturated. The values for the oxygen in the blood from an arm vein have not differed materially from the values obtained from the average blood flowing to the right side of the heart. A similar value has been obtained by calculation in the experiments on the blood flow done by Krogh and Lindhard's (11) method.<sup>4</sup> In animals very varying figures have been obtained in blood from different organs (Barcroft, 7).

However, our knowledge has been only approximate. Great variations occur; how great, or how caused, we do not know. It is therefore necessary to establish the limits in not too small a number

<sup>3</sup>The term "consumption" means the percentage of oxygen absorbed in the circulation. The arterial blood was considered saturated with oxygen.

<sup>4</sup>See discussion of blood flow experiments in Paper II, *J. Exp. Med.*, 1918, xxvii, in press.

of normal individuals before we can hope to be able to interpret results from abnormal individuals. This paper is a report of thirty-eight determinations on twenty normal individuals.

### *Technique.*

Two operations are involved: first, the drawing of a sample of venous blood; and second, the determination of its oxygen content. The determination of the oxygen has been done by Van Slyke's method (12). This method is quicker, easier to handle, and more exact than the Haldane-Barcroft, where the great volume of foreign air in the apparatus requires elaborate facilities for keeping the temperature constant. A determination with Van Slyke's apparatus (including cleaning of the machine) requires 5 to 10 minutes. The figure can be read with an accuracy of 0.004 cc. Adding to this the inevitable error in measuring and delivering 2 cc. with a pipette, we can say that the difference between duplicates can always be kept within 0.01 cc. of oxygen, which is 0.5 volume per cent of the 2 cc. of blood analyzed. As will be seen from the tables, duplicates usually agree much more closely. Greater differences are caused only by gross errors, such as by imperfect mixture of the corpuscles in the blood, or by allowing time for a slow oxidation (or deoxidation) of the blood between the two determinations (see below).

The other part of the technique, the obtaining of a sample of blood, has been as follows: The arm from which the blood is drawn rests comfortably at the side of the body on a small, moderately soft pillow. The blood is drawn without stasis and the pulse and respiration are counted from the moment the blood appears.<sup>5</sup> The apparatus for blood drawing consists of a very sharp needle connected by means of a 3 to 4 cm. rubber tube to a glass pipette of about 0.5 cm. bore. The pipette and the rubber have a film of sodium oxalate crystals in them. This is obtained by wetting the interior with a saturated solution of oxalate, which is dried with a current

<sup>5</sup> It might be more logical to start counting the pulse a half minute before the blood appears. However, the pulse rate usually keeps almost constant under the procedure if it is skilfully done.

of air. In drawing the blood one puts the upper end of the pipette into the mouth (some cotton wool will prevent saliva from flowing down) and quickly introduces the needle in the proximal direction. After a little practice it is generally possible to obtain blood at the first puncture. If the needle is dull, unnecessary pain will be caused and the vein will probably escape the needle. If the point is too long, it will almost invariably hit the opposite wall of the vein before the point is entirely within. The needle is introduced best at an angle of about  $50^{\circ}$  with the surface of the arm. The opening of the needle must be kept downward or to the side so that the upper wall of the vein will not close the opening when the blood is being drawn. In drawing the blood only a gentle suction is necessary, and if air should happen to be sucked through the blood the sample must be thrown away and a new pipette used. The needle and rubber can often be kept *in situ* while another pipette is connected with the rubber. When a sufficient amount of blood (6 to 12 cc.) has been drawn, the upper end of the pipette is closed by a finger and the needle withdrawn. Then the needle and the rubber are taken away from the pipette and the blood is discharged into a cylinder (2 cm. wide and with a few oxalate crystals in the bottom) below a layer of white mineral oil at least 2 cm. deep. Just before the last 0.5 to 1 cc. of blood has run out, the pipette is again closed with a finger and withdrawn, because the upper part of the blood in the glass pipette has been oxidized and must be discarded. The blood in the cylinder is then stirred up with a glass rod to secure a good mixing with the oxalate. From the cylinder 2 cc. samples of blood are introduced into Van Slyke's apparatus below a layer of evacuated dilute ammonia as described by Van Slyke (12). Before a sample is taken the blood must be stirred up carefully to secure homogeneity. If the blood has been kept in an ice box for some time (see below) it is often difficult to secure homogeneity, even after it has gained room temperature. It gets more sticky and adheres to the sides of the cylinder. It is therefore advisable to do the determinations on fresh blood. After the blood has been stirred with a rod, a 2 cc. pipette is introduced, the upper end being closed to prevent mineral oil from coming up, and after a few seconds' additional stirring with the pipette, the sample is sucked up and dis-



charged into the apparatus. The pipette must be calibrated for 2 cc. outflow, and may be calibrated either for complete discharge or for discharge to a mark on the lower stem. The latter is preferable, but the pipette for complete discharge gives in practice equally good results, because only a very little surface of blood touches the air, so the whole blood column may be used without introducing any appreciable error. The calculation of the amount of oxygen in the sample is simple (see p. 473, Van Slyke's paper (12)). The determination of the total oxygen-combining power of the blood has in some instances been done with the Van Slyke apparatus, in other instances with Palmer's colorimetric method (13). If the blood has been kept in an ice box for some time, it may be impossible to rely upon the homogeneity. Consequently when the main sample of blood is placed under an oil layer, a portion of about 1 or 2 cc. is transferred to a special dish without oil and used for the Palmer determination. It is advisable to have a glass bead in this dish to stir the sample.

#### RESULTS.

The results are given in Tables I and II. Besides the date and hour of bleeding and of the determination, the pulse and respiration during the bleeding, three groups of figures are given. (1) The oxygen content of the venous blood in volume per cent corrected for temperature and pressure. In all instances double determinations have been done and the average has been taken. (2) The oxygen-combining power of the blood (hemoglobin), in most instances calculated from the determination of the hemoglobin by Palmer's method.<sup>6</sup> (3) The difference between the oxygen in the venous blood and the total oxygen-combining power of the hemoglobin.

This last figure is important. It is difficult to attach much significance to the figure for the venous oxygen, unless we know what percentage of the total oxygen-combining power of the hemoglobin the venous oxygen represents. The venous oxygen uncontrolled by hemoglobin determinations is as incomplete as a nitrogen excretion considered without relationship to the nitrogen intake. We have

<sup>6</sup> I am greatly indebted to Dr. Walter W. Palmer, who has done the majority of these determinations.

termed the difference between the venous oxygen and the total oxygen-combining power of the hemoglobin the *oxygen unsaturation* of the venous blood since it represents that portion of the hemoglobin which does not have its oxygen-binding values saturated. The term unsaturation is used in a sense analogous to that in which it is used in organic chemistry, defined by Webster as "falling short of saturation, not combined to the greatest possible extent."<sup>7</sup>

Thirty-eight determinations on twelve different normal individuals have been done. In Table I are given twenty determinations on one person, and in Table II eighteen determinations on eleven different people. Care has been taken to draw the blood from a person resting as completely as possible, because we know that an increase in the metabolism necessarily is followed by an increased oxygen consumption. Before the drawing of the blood the subjects rested on a couch for some time, in some experiments 10 minutes, in others half an hour. In some instances the blood was taken in the morning, before getting up. It does not seem to make any difference whether the resting period is 10 minutes or 30. The oxygen content of the blood taken in the morning had a tendency to be low, particularly when the person had been awake only a few seconds. The average figure for the oxygen content in the twenty determinations on No. 1 is 13.7 volume per cent, with a maximum of 16.84 volume per cent and a minimum of 9.55. The average figure for the eighteen determinations on the other cases is 13.6 volume per cent, with a maximum of 17.98 and a minimum of 10.36 volume per cent.

The total oxygen-combining power (the hemoglobin) has varied from 21.44 (hemoglobin 116 per cent) to 17.50 (hemoglobin 94.5 per cent). This must, of course, influence the amount of oxygen in the venous blood. In the determination in No. 27, for instance,

<sup>7</sup> The term oxygen consumption has been applied to the figure for which we prefer the more exact term unsaturation. The use of consumption in this connection implies that all the venous oxygen which the venous blood requires in order to be saturated is lacking for the reason that it has been consumed by the tissues. This assumption would be correct if one could always be certain that the arterial hemoglobin is 100 per cent saturated with oxygen. Since one cannot be certain of this, it seems preferable to use the term unsaturation, which is free from the above assumption.

TABLE I.  
*Determinations in Venous Arm Blood of Oxygen, Hemoglobin, and Oxygen Unsaturation in a Normal Subject.*

Determination.	Bleeding.			Oxygen content of venous blood.					Hemoglobin (Palmer method).	Calculated oxygen capacity.	Difference between oxygen capacity and oxygen in venous blood = oxygen unsaturation.	Pulse.	Respirations.	Remarks.	
	Date.		Hour.	Arm.	Sample 1.		Sample 2.								Average.
					Hour.	Vol. per cent.	Hour.	Vol. per cent.							
1	1917 Jan. 25	3 30	Right.	4 20	14.20	4 50	14.22	14.22	108	19.96	5.75	78	14	10 min. rest.	
2	" 26	3 20	"	3 25	13.95	5 25	14.20	14.26	—	(19.96)	5.83	74	12	"	
3	" 27	4 30	"	4 35	14.50	5 10	14.50	14.50	—	(19.96)	5.42	86	20	"	
4	" 29	12 10	Left.	12 30	13.42	12 50	13.42	13.42	—	(19.96)	6.54	70	16	"	
5	" 30	4 30	"	4 40	15.40	4 50	15.20	15.30	108	19.96*	4.66	90	14	"	
6	" 31	12 20	Right.	12 40	15.80	3 15	15.75	15.78	—	(19.96)	4.18	78	14	"	
7	Feb. 6	10 40	"	10 50	16.80	11 10	16.88	16.84	108	19.96	3.12	80	14	"	
8	" 6	12 10	"	12 20	12.90	12 35	13.22	13.06	—	(19.96)	6.90	70	14	"	
9	" 13	5 10	"	5 15	13.64	5 30	13.64	13.64	110	20.34	6.70	71	14	"	
10	" 28	12 20	Left.	12 30	14.25	12 50	14.28	14.27	—	(18.83)	4.56	73	11	½ hr. rest.	
11	Mar. 1	11 30	"	11 55	16.15	12 20	16.11	16.13	—	18.83*	2.70	78	12	"	
12	Apr. 4	11 00	Right.	12 10	15.06	12 30	14.35	14.70	100	18.50	3.74	72	14	"	
13	May 30	7 15	"	7 35	9.54	7 55	9.56	9.55	—	(18.50)	8.95	—	—	Before getting up. Just awakened.	
14	" 31	7 15	"	7 30	15.02	7 45	14.99	15.01	100	18.50	3.49	76	14	Before getting up. Had been awake for ½ hour.	
15	June 1	7 15	"	7 50	13.97	8 30	14.01	13.99	—	(18.50)	4.51	80	16	Awake for ½ hr.	
16	" 2	7 15	"	7 30	12.34	7 45	12.37	12.36	—	(18.50)	6.14	72	14	" " ½ "	
17	" 3	7 30	"	7 45	9.79	8 10	10.74	10.27	100	18.50	8.23	66	12	Deep sleep just before blood was taken.	
18	" 4	7 15	Left.	7 30	12.54	11 00	12.49	12.52	—	(18.50)	5.98	70	12	Before getting up. Had been awake for ½ hr.	
19	" 6	7 15	"	10 15	12.24	11 45	12.20	42.22	102	18.87	6.65	68	12	Awake for ½ hr.	
20	" 7	7 15	Right.	7 30	12.23	7 45	11.97	12.10	102	18.87	6.77	70	14	" " ½ "	

\* Determination of the total oxygen-combining power of the blood done by the Van Slyke apparatus.

TABLE II.  
*Determinations in Venous Arm Blood of Oxygen, Hemoglobin, and Oxygen Unsaturation in Eleven Normal Subjects.*

Determination.	Bleeding.			Oxygen content of venous blood.							Hemoglobin (Folmer method) per cent.	Calculated total oxygen capacity.	Difference between oxygen capacity and oxygen in venous blood = oxygen unsaturation.	Pulse.	Respirations.	Remarks.	Name.
	Date.	Hour.		Arm.	Sample 1.		Sample 2.		Average.								
		Hour.	Vol. per cent.		Hour.	Vol. per cent.											
	1917																
21	Feb. 1	12.10	Right.	12.20	15.50	12.40	15.42	15.37	115	21.26	5.89	70	14	10 min. rest.	Dr. St.		
22	" 7	11.40	Left.	11.50	16.03	12.10	16.24	16.14	116	21.44	5.30	84	13	10 " "			
23	" 7	12.40	"	12.50	16.76	1.10	16.66	16.71	116	21.44	4.73	81	14	10 " "			
24	Feb. 8	12.15	Left.	12.25	11.66	3.00	11.98	11.82	—	17.48*	5.66	55	12	10 min. rest.	Dr. F.		
25	Apr. 9	12.00	"	3.20	12.27	3.30	12.25	12.26	99	18.48	6.22	68	—	10 " "			
26	" 30	12.00	Right.	12.30	13.17	1.00	13.04	13.11	100	18.50	5.39	58	19	½ hr. rest.			
27	Feb. 5	11.20	Left.	11.40	18.01	11.55	17.95	17.98	113	21.11*	3.13	68	14	10 min. rest.	Dr. V. S.		
28	" 5	12.40	"	12.50	15.82	1.10	16.25	16.04	—	—	5.07	—	—	10 " "			
29	Feb. 9	10.00	Right.	10.10	13.93	10.25	14.15	14.04	101	18.86	4.82	78	14	10 min. rest.	Dr. Bl.		
30	" 9	1.15	"	1.30	12.35	2.10	12.61	12.48	—	18.86	6.38	76	14	10 " "			

31	June 3	11.15	Left.	11.30	12.00	12.15	12.00	12.00	108	20.00	8.00	54	22	$\frac{1}{2}$ hr. rest.	Dr. L.
32	" 14	3.30	"	3.50	10.62	4.10	10.68	10.65	98.4	18.30	7.65	52	20	$\frac{1}{2}$ " "	
33	Feb. 2	10.45	Right.	10.55	13.15	11.10	13.10	13.13	107	19.80*	6.67	70	18	10 min. rest.	T
34	Feb. 1	4.20	Left.	4.30	15.55	4.45	15.45	15.50	100	18.50	3.00	88	18	10 min. rest.	R
35	May 17	10.30	Right.	11.00	12.48	11.30	12.74	12.61	105	19.40	6.79	62	24	$\frac{1}{2}$ hr. rest.	Dr. D.
36	June 6	10.40	Left.	11.00	10.75	11.30	11.15	10.95	94.5	17.50	6.55	70	—	$\frac{1}{2}$ hr. rest.	Miss S.
37	June 8	7.15	Left.	7.30	10.40	7.45	10.32	10.36	103	19.04	8.68	78	—	Before getting up.	Dr. P.
38	June 13	7.00	Left.	7.15	11.52	7.25	11.56	11.54	110	20.37	8.83	70	14	Before getting up.	Dr. M.

\* Determination of the total oxygen-combining power of the blood done by the Van Slyke apparatus.

TABLE III.  
*Determinations of Oxygen in Venous Blood Kept for Varying Periods under a Layer of Mineral Oil.*

Name.	No. in Tables I and II.	Bleeding.		Determination.		Oxygen content of venous blood.	Remarks.
		Date.	Hour.	Date.	Hour.		
L.	2	1917		1917		<i>vol. per cent</i>	
		Jan. 26	3.20 p.m.	Jan. 26	3.25 p.m.	13.95	5 min. in laboratory.
		" 26		" 26	5.25 "	14.24	3 hrs. " refrigerator at 6°.
		" 27		" 27	12.00 n.	14.20	18½ " " " 6°.
L.	3			" 27	3.30 p.m.	12.28	3½ " " laboratory " 24°.
		Feb. 27		Feb. 27	4.35 p.m.	14.50	5 min. in laboratory at 24°.
		" 27		" 27	5.10 "	14.50	35 " " " 24°.
		" 28		" 28	10.30 a.m.	14.60	41 hrs. " refrigerator " 6°.
I.	6			Jan. 31	12.40 p.m.	15.80	20 min. in laboratory at 23°.
		Jan. 31	12.20 p.m.	" 31	3.15 "	15.75	2½ hrs. " " 23°.
W.	(2) From an- other publica- tion.			Mar. 24	2.30 p.m.	7.68	2½ hrs. in laboratory at 23°.
		Mar. 24	12.00 n.	" 24	2.50 "	7.66	20 min. " " 23°.
		" 27		" 27	3.40 "	11.11	73 hrs. in refrigerator " 6°.
L.	(1) From an- other publica- tion.			July 2	2.10 p.m.	17.14	10 min. in laboratory at 25°.
		July 2	2.00 p.m.	" 2	2.30 "	17.32	10 " " " 25°.
		" 9		" 9	3.00 "	19.05	7 days in ice box.
L.	(2) From an- other publica- tion.			July 3	10.10 a.m.	17.63	20 min. in laboratory at 26°.
		July 3	9.50 a.m.	" 3	10.30 "	17.86	20 " " " 26°.
		" 7		" 7	2.00 p.m.	18.64	4 days in ice box.
T.	(4) From an- other publica- tion.			July 13	11.10 a.m.	13.83	1½ hrs. in laboratory at 27°.
		July 13	9.30 a.m.	" 13	3.40 p.m.	13.99	4½ " " ice box.
		" 13		" 13	9.30 "	13.82	5 " " laboratory.

the amount of oxygen in the venous blood is greater than the total capacity of the hemoglobin in No. 36. Such measures illustrate the necessity of taking as a measure of oxygen consumption the oxygen unsaturation rather than the oxygen content of the venous blood. The average value of the oxygen unsaturation for Case 1 is 5.5 volume per cent, the minimum being 2.70, the maximum 8.95. The average for the eighteen determinations on Cases 2 to 12 is 6.0 volume per cent, with a minimum of 3.00, a maximum of 8.83 (Table IV).

The determinations are too few to allow an interpretation of the different causes of the variations.<sup>8</sup> Series of determinations on different individuals under different conditions might throw light upon that problem. There is generally a decrease in the oxygen unsaturation with increasing pulse rate, but it is by no means invariable. It is worth mentioning that a value for the oxygen unsaturation of more than 8 volume per cent is found in only four instances, in all of which the blood was drawn in the morning, a few seconds after the subject awoke. In other words, the highest degree of unsaturation is found under circumstances where the metabolism is lowest, when the individual usually has his lowest pulse rate, and when all exciting impressions have been excluded for a considerable time.

In some instances it has been impossible to do the blood analysis immediately after a bleeding. In order to find out how long, and under what conditions the blood samples can be stored, experiments have been done as shown in Table III.

The figures show that the blood can be kept in an ice box at a low temperature (6°C.) for a considerable length of time (at least 24 hours) before any appreciable changes take place. After a certain time the blood will absorb oxygen through the oil and the values will increase. The opposite happens when the blood is kept in the laboratory. We cannot expect it to keep constant more than 2 hours. After that interval the oxygen content diminished rapidly, probably on account of bacterial action.

<sup>8</sup> A series of papers in which the technique described in this paper is applied to the clinical study of patients with heart disease will be published in *The Journal of Experimental Medicine*.

## SUMMARY.

1. A report is made of a series of determinations by the Van Slyke method of the oxygen in the blood drawn from the vena mediana in normal resting individuals.

2. A procedure for drawing the blood without stasis or absorption of air has been devised.

3. The difference between the total oxygen-combining power of the hemoglobin and the oxygen in the venous blood is calculated. This figure is termed the oxygen unsaturation of the venous blood.

4. The results are the following:

TABLE IV.

No. of individuals.	No. of determinations.	Oxygen content of venous blood. Vol. per cent.			Oxygen unsaturation of venous blood. Vol. per cent.		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
12	38	17.98	9.55	13.6	8.95	2.70	5.8

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## STUDIES OF OXYGEN IN THE VENOUS BLOOD.

### II. STUDIES OF THE OXYGEN UNSATURATION IN THE VENOUS BLOOD OF A GROUP OF PATIENTS WITH CIRCULATORY DISTURBANCES.

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#### INTRODUCTION.

In a previous paper (1) a report was made of a series of determinations of the oxygen content of the venous blood from the vena mediana cubiti of twelve normal resting individuals. The difference between the oxygen in the venous blood and the total oxygen capacity of the hemoglobin (determined by Van Slyke's method (2) or Palmer's method (3) ) was called the oxygen unsaturation of the venous blood. In this way the differences in hemoglobin concentration are eliminated.<sup>1</sup> It was found that the extent of oxygen unsaturation of the venous blood in normal individuals fell between 2.5 and 9 cc. per 100 cc. of blood. However, values above 8 volume per cent were only met with under special conditions; namely, when the blood was drawn in the morning immediately after the subject had been awakened from sleep. The blood was drawn from an arm vein without any stasis whatever, after the subject had rested for a half hour on a couch or bed. A sharp, not too pointed, needle was connected with a rubber tube to a glass pipette, 25 to 30 cm. long and  $\frac{1}{2}$  cm. wide, which had a film of oxalate on the inside.<sup>2</sup> The blood (about 10 cc.) was sucked

<sup>1</sup> A determination of the oxygen in the venous blood without a simultaneous determination of the total oxygen capacity is just as incomplete as a determination of the urea in the blood or the nitrogen in the urine, without considering the intake.

<sup>2</sup> This was obtained by wetting the tube and rubber with a concentrated solution of oxalate and drying by an air current.

TABLE I.  
*Patients with Circulatory Disturbances Compensated at Rest.*  
*Experimental.*

Case No.	Determination No.	Age	Bleeding.			Oxygen content of venous blood.								Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a - v).	Pulse.	Respirations.
			Date.	Hour.	Condition.	Sample 1.		Sample 2.		Average (v).									
						Hour.	Result.	Hour.	Result.										
1	1	41 yrs.	1917 Feb. 3	11.00 a.m.	1 hr.	11.20	17.72	11.30	18.70	18.21	123	22.70	21.96 22.34	4.49	98	18			
			May 22	10.30 a.m.	In bed.	11.35	12.98	11.55	13.50	13.24	100	18.50		5.26	80	22			
3	3	47	Feb. 12	4.00 p.m.	5 min.	4.15	15.30	4.25	15.14	15.22	100	18.50	18.74 18.00	3.28	84	24			
			May 6	2.30 "	1 hr.	2.50	14.24	3.15	14.26	14.25			16.90 17.10	2.75	100	22			
4	5	29	May 8	2.10 p.m.	In bed.	2.40	5.44	3.00	5.42	5.43	44.5	8.23		2.80	84	22			
5	6	49	May 22	9.20 a.m.	In bed.	10.50	9.19	11.08	9.35	9.27	92	17.02		7.75	80	22			
	7		June 21	11.00 "	" "	11.45	12.24	12.00	12.24	12.24	89.5	16.54		4.30	80	20			
	8		" 27	9.30 "	$\frac{1}{2}$ hr.	10.30	11.88		11.46	11.67	88.5	16.37		4.70					
	9		" 30	10.20 "	$\frac{1}{2}$ "	10.35	10.89	10.55	10.93	10.91	88.5	16.37		5.46	80	20			
6	10	22	June 26	9.50 a.m.	$\frac{1}{2}$ hr.	10.50	11.78	11.20	12.04	11.91	103	19.06		7.15	82	20			
7	11	25	Apr. 18	12 n.	In bed.	12.40	13.52	12.55	13.60	13.56	111	20.53		6.97	76	24			
8	12	37	Feb. 12	12 n.	In bed.	12.15	15.30			15.30	111	20.53		5.23	75	18			
9	13	31	Feb. 17	3.00 p.m.	In bed.	3.50	17.15	4.15	16.90	17.03	114	21.10		4.07	67	26			
	14		Mar. 8	10.00 a.m.	" "	11.10	14.62	11.40	14.42	14.52	106	19.60		5.08	90	22			
10	15	57	June 27	12.30 p.m.	$\frac{1}{2}$ hr.	2.10	12.89	2.30	12.47	12.68	105	19.44		6.76	96	28			
11	16	25	May 31	3.00 p.m.	$\frac{1}{2}$ hr.	4.00	11.65			11.65	89	16.46		4.81	56	28			
12	17	18	Feb. 28	10.30 a.m.	In bed.	10.55	6.72	11.10	6.75	6.74	71.4	13.20		6.46	80	22			

*Clinical.*

Case No.	Determining No.	Oxygen unsaturation (a - v). vol. per cent	Diagnosis and clinical notes.
1	1	4.49	Arteriosclerosis; hypertensio (blood pressure 160-96); hypertrophy of heart; shortness of breath on exertion.
2	2	5.26	Arteriosclerosis; hypertension; hypertrophy of heart (blood pressure 296-130); some moist râles in both lungs.
3	3 4	3.28 2.75	Chronic interstitial nephritis; hypertension (blood pressure 180-130); hypertrophy of heart; shortness of breath on exertion.
4	5	2.80	Chronic interstitial nephritis; chronic uremia; hypertension (blood pressure 195-133); hypertrophy of heart; edema; ascites; stasis in lungs (edema); urea retention in blood.
	6	7.75	Chronic nephritis; hypertension; cardiac hypertrophy (he was never incompensated at rest. He had shortness of breath on slight exercise).
5	7	4.30	May 22. A few râles at base of lungs; no dullness. Blood pressure 218-126.
	8	4.70	June 21. No râles. Blood pressure 214-108.
	9	5.46	" 27. Pains behind sternum. No râles. Blood pressure 220-112.
6	10	7.15	" 30. No râles.
7	11	6.97	Aortic insufficiency; hypertrophy of heart (blood pressure 300-90).
8	12	5.23	Mitral stenosis; auricular fibrillation.
9	13 14	4.07 5.08	Mitral stenosis and insufficiency; auricular fibrillation.
10	15	6.76	Mitral insufficiency; auricular fibrillation.
11	16	4.81	Auricular fibrillation; chronic myocarditis.
12	17	6.46	Chronic myocarditis. Pulse regular.
			Pericarditis; some râles at bases of lungs.

\* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

TABLE II.  
*Patients with Incompensated Heart Failure.*  
*Experimental.*

Case No.	Determination No.	Age.	Bleeding.			Oxygen content of venous blood.								Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a - v).	Pulse.	Respirations.
			Date.	Hour.	Condition.	Sample 1.		Sample 2.		Average (v).									
						Hour.	Result.	Hour.	Result.		vol. per cent.								
13	18	30	1917 Feb. 10	11.00 a.m.	1½ hr.	11.20	6.56	11.35	6.50	6.53	93	17.18	16.65	16.69	10.14				
	19		" 19	10.05 "	In bed.	10.30	6.67	1.45	6.58	6.63	100	18.50			11.87	62	22		
14	20	57	Feb. 9	3.10 p.m.	In bed.	3.30	8.60	3.50	8.84	8.72			22.52	22.46	13.76	96	36		
	21		" 19	10.00 a.m.	" "	10.10	5.93	10.50	5.79	5.86	112	20.71			14.85	86	32		
	22		Apr. 27	2.40 p.m.	" "	2.45	12.39	3.10	12.55	12.47	120	22.20			9.73	86	32		
	23		June 26	10.00 a.m.	" "	10.10	7.16	10.40	7.62	7.39	106	19.61			12.22	77	24		
15	24	64	Feb. 21	11.10 a.m.	In bed.	11.20	12.12	11.30	12.05	12.07	119	22.00			9.91	36	20		
	25		Mar. 24	12 n.	" "	2.30	7.68	2.50	7.66	7.67	117	21.65			13.98	28	20		
	26		Apr. 21	11.00 a.m.	" "	2.10	10.48	2.30	10.46	10.47	110	20.36			9.89	32	24		

*Clinical.*

Case No.	Determination No.	Oxygen unsaturation (a-v).	Diagnosis and clinical notes.
		<i>vol. per cent</i>	
13	18	10.14	Mitral stenosis and insufficiency; auricular fibrillation; adherent pericardium. Rales at base of lungs; cyanosis of face; distention of veins in neck; enlargement of liver; ascites; low diuresis.
	19	11.87	
14	20	13.76	Mitral insufficiency; arteriosclerosis; hypertrophy of heart.
	21	14.85	Feb. 9. Moist rales in both lungs; severe cyanosis; enlargement of liver; severe edema of legs.
	22	9.73	" 19. Same condition.
	23	12.22	Apr. 27. Has improved. Very slight cyanosis; no edema; marked enlargement of liver Still some rales in lungs.
15	24	9.91	June 26. Slight edema of legs; slight cyanosis. Still rales at base of lungs.
			Heart block; syphilis of heart.
			Feb. 21. Slight cyanosis of lips; slight jaundice of sclerae and skin; rales in lungs; no edema; great enlargement of liver.
	25	13.98	Mar. 24. Same condition.
	26	9.89	Apr. 21. " "

TABLE III.

*A Patient with Incompensated Heart Failure. Oxygen Determined by Barcroft's Method.*

*Experimental.*

Case No.	Determination No.	Age.	Bleeding.		Oxygen content of venous blood.			Total oxygen capacity (Barcroft's method(a)).	Oxygen unsaturation (a-v).	Pulse.	Respirations.
			Date.	Condition.	Sample 1.	Sample 2.	Average (v).				
		yrs.	1916-17		vol. per cent	vol. per cent	vol. per cent	vol. per cent	vol. per cent		
16	27	17	Oct. 25	In bed.	2.40		2.40	15.30	12.90		
	28		Nov. 20	" "	2.48		2.48	17.20	14.72	120	40
	29		" 21	" "	3.36	1.36	2.36	17.56	15.20	122	40
	30		" 22	" "	2.40		2.40	17.56	15.16	124	44
	31		Jan. 12	" "	6.86		6.86	17.20	10.34	120	30

*Clinical.*

Case No.	Determination No.	Oxygen unsaturation (a-v).	Diagnosis and clinical notes.
		vol. per cent	
16	27	12.90	Mitral and aortic insufficiency.
			Oct. 25, 1916. Moderate cyanosis; few râles at base of lungs.
	28	14.72	Nov. 20. Lungs clear.
	29	15.20	" 21. Cyanosis.
	30	15.16	" 22. Enlargement of liver.
	31	10.34	Jan. 12, 1917. Less cyanosis; no râles; slight enlargement of liver.

up in the pipette, from which it was discharged into a cylinder, 2 cm. in diameter, below a layer (2 cm.) of mineral oil to prevent oxidation. The last (upper) 1 or 2 cc. of blood, a part of which had been oxidized, were put in a separate dish and used for a Palmer determination of the hemoglobin. Samples of blood for a Van Slyke determination were taken from the cylinder after careful stirring.<sup>3</sup>

*Observations on Patients.*

This paper is a report of a preliminary investigation on the oxygen unsaturation in patients with circulatory disturbances. Thirty-

<sup>3</sup> For further technical details see Paper I of this series (1).

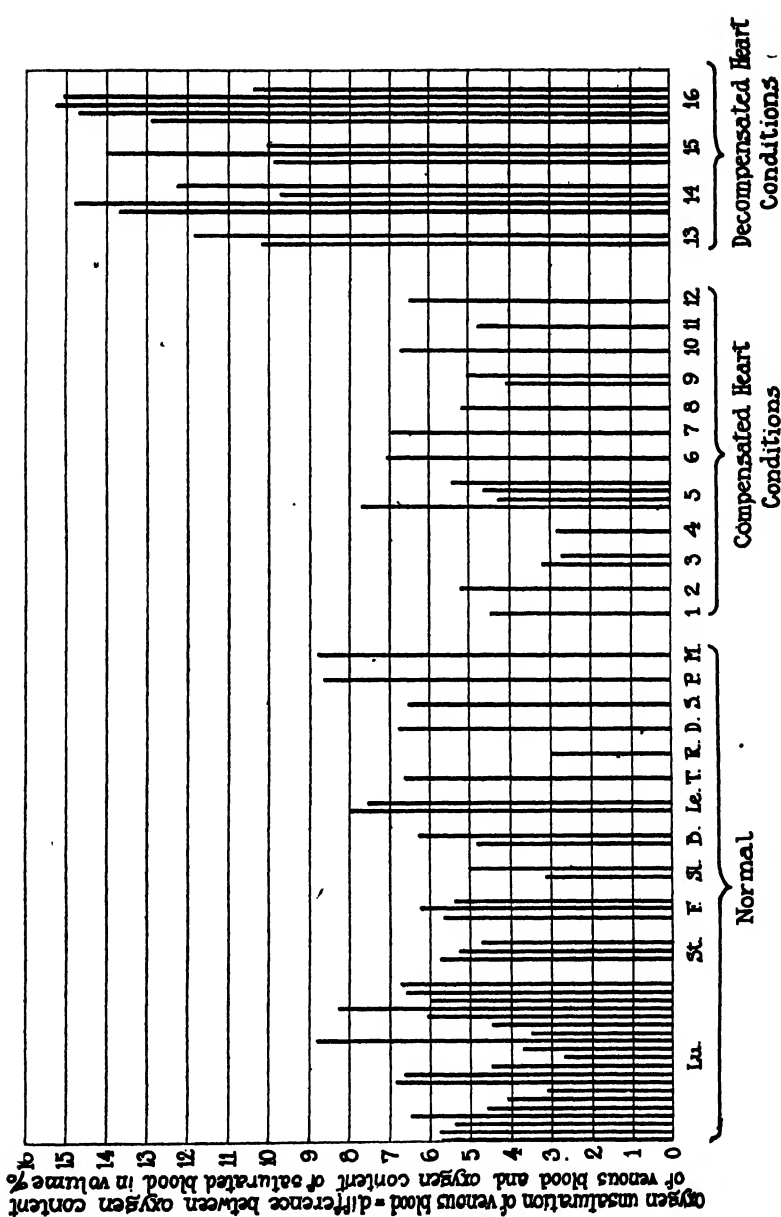
one determinations were made on sixteen patients with different kinds of circulatory disturbances. The procedure in preparing the patients and the technique in drawing the blood have been exactly as described in the first paper. Van Slyke's method (2) has been used for the determination of the oxygen in all but one case (No. 16 in Table III) where Barcroft's method has been used.

The patients have been divided into two groups according to the condition of the circulation. Results and clinical notes from patients with clinically compensated heart lesions have been tabulated in Table I. The data from the cases with uncompensated circulatory conditions are collected in Tables II and III. As in Paper I, we have tabulated both the oxygen content of the venous blood samples and the extent of the oxygen unsaturation.

One case (No. 4, Table I) is particularly worth mentioning, because it shows the importance of using the oxygen unsaturation and not the absolute value of the venous oxygen alone in comparing the results from different individuals. This patient was brought to the hospital in a very sick condition. He was suffering from dyspnea and palpitation and had ascites and severe edema of the legs. The oxygen in his venous blood was 5.43 volume per cent, which was much lower than any figure obtained on normal individuals, and even lower than most of the figures obtained from patients with uncompensated heart disturbances. The total oxygen-combining power of his blood was very low, however, 8.23 volume per cent, instead of the usual average 18.50 volume per cent. The oxygen unsaturation of his venous blood is therefore not only within normal limits but even rather low. The clinical diagnosis was chronic nephritis, chronic uremia, hypertension, hypertrophy of the heart, anemia, and urea retention. The edema was considered to be entirely of nephritic origin. Therefore, in respect to both the clinical picture and the findings in the blood, he is to be grouped in Table I, with patients free from uncompensated circulatory disturbances. On the other hand, we may find a high figure for the venous oxygen combined with a high extent of unsaturation (No. 13, Table II) on account of a high content of hemoglobin.

Text-fig. 1 is a diagrammatic representation of the figures for the oxygen unsaturation in normal individuals<sup>4</sup> and in the patients re-

<sup>4</sup> Reported in Paper I (1).



TEXT-FIG. 1. Oxygen unsaturation of venous blood in a group of normal subjects<sup>4</sup> and of patients reported in the present paper.



ported in the present paper. It will be seen that the upper limit for the unsaturation in normal individuals (9 volume per cent) is not exceeded by any figure obtained from patients belonging to the clinically compensated group. The figures are all distributed in the same haphazard manner over the area between 2.5 and 9 volume per cent.

The figures from the uncompensated group show quite another picture. They are all above the upper normal limit, lying between 9.7 and 15.2 volume per cent.

No attempt has been made to subdivide the cases according to the anatomical or physiological form of the heart lesion. There may be a difference, but the cases and the number of determinations in each case are much too few to allow any such attempt. The only statement which can be made is that the oxygen unsaturation of the venous blood from patients with clinically compensated heart conditions has been found within the normal limits, whereas the oxygen unsaturation in some patients with uncompensated heart lesions has been above the upper normal limit. It is probably not even justifiable to make this a general statement. It seems probable that patients with non-stationary conditions might fall outside this rule. We might suppose that a patient with an uncompensated, but improving heart lesion could show normal figures. On the other hand, a patient with a heart lesion where uncompensation is developing might possibly show increased oxygen unsaturation before the ordinary clinical symptoms had developed. If that should be true, the determination of the oxygen unsaturation would be of great clinical significance. Series of determinations on a single patient might throw light on this question.

#### DISCUSSION

There is extensive clinical and experimental evidence for assuming that the condition of the heart has a certain influence on the extent of oxygen unsaturation of the venous blood. A previous investigation on the blood flow (minute volume) in patients with uncompensated heart lesions (4) has shown a considerable decrease in the minute volume of the heart compared with that found in normal individuals

(5-14), and in most cases of compensated heart lesions<sup>5</sup> (4, 6, 11, 15): The figures obtained by Stewart (16) by determining the blood flow in the hands and feet point in the same direction. A retarded circulation, the rate of oxygen consumption not being decreased, may logically be assumed to result in a necessarily increased oxygen unsaturation of the average venous blood. The question is whether one is justified in assuming a retarded circulation from an increased oxygen unsaturation in the blood drawn from the vena mediana cubiti or from another superficial vein..

We know that other factors than the output from the heart may influence the extent of unsaturation in the blood from an arm vein. These factors are<sup>6</sup> (1) the possible unsaturation of the arterial blood; (2) variations in the metabolism of the tissues drained by the vein tapped as compared with the rest of the body; (3) variations in the rate of blood flow through the tissues drained compared with the rest of the body.

We are unfortunately, for the time being, only to a limited extent able to measure, control, or eliminate the influence of these factors.

(1) The dissociation curve for oxyhemoglobin shows that the blood must become nearly saturated (usually about 96 to 98 per cent) during the passage through the lungs, provided that all the blood flowing through comes into equilibrium with the alveolar air. This has been proved experimentally on animals by several investigators, and in

<sup>5</sup> Plesch (6) and Means and Newburgh (11) found normal figures for the blood flow in all their patients with compensated heart lesions, whereas Lundsgaard (4) found diminished minute volume not only in uncompensated cases but in some patients with compensated heart conditions (mitral stenosis and auricular fibrillation), which agrees with Stewart's (16) observations for the local blood flow.

<sup>6</sup> One condition which might theoretically affect the oxygen saturation of the hemoglobin in arterial blood is uncompensated acidosis, defined by Hasselbalch and Gammeltoft (17) and Van Slyke and Cullen (18), in which increased free carbonic acid causes an increase in the actual hydrogen ion concentration of the blood. As shown by Barcroft (19) this increase reduces the percentage oxygen saturation of hemoglobin under a given oxygen tension. Since, however, the differences thus caused in oxygen saturation become considerable only under reduced oxygen tension, it appears improbable that this factor, even in the infrequently occurring uncompensated acidosis, exerts a significant effect on the degree of saturation of the arterial hemoglobin.

man it has been investigated by Hürter (20). By drawing blood from a radial artery and using Barcroft's method for determination of the oxygen, he found in four normal individuals a saturation of 94, 92, 99, and 100 per cent. In four patients with compensated heart lesions the saturation was 90, 100, 90, and 95 per cent. In one patient with uncompensated aortic insufficiency and stasis bronchitis in the lungs the saturation was 81 per cent. In another patient with uncompensation (Pick's disease) it was 92 per cent. A patient with patent ductus Botalli showed 88 per cent saturation. One with diffuse bronchitis showed 94 per cent, and another with phthisis, 88 per cent. Two patients with lobar pneumonia showed 81 and 79 per cent saturation. Barcroft<sup>7</sup> found a saturation of 94 per cent in a normal individual.

In several of my patients with compensated heart lesions moist râles have been heard at the base of the lungs. This circumstance does not seem to have influenced substantially the amount of oxygen in the venous blood. These râles are probably to a great extent just a proof that the air really does go down into the alveoli of the lowest part of the lungs. The whole problem is not sufficiently investigated to allow a decision concerning the influence of the involvement of the lungs in the individual case. We may confine ourselves to making a careful examination of the lungs in every case, hoping that special investigation of the unsaturation in patients with lung diseases or experimental studies will give us sufficient information.

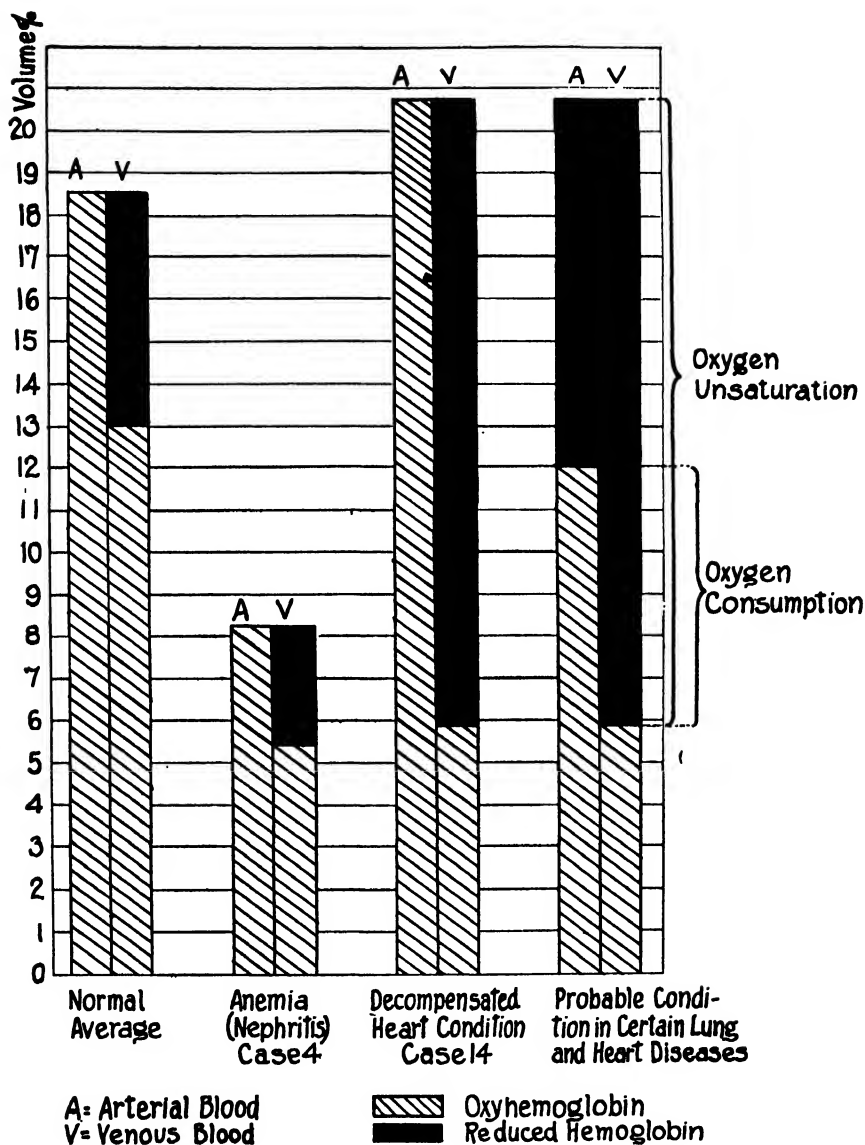
(2) The variations in the metabolism are presumably minimized by performing the experiments under definite conditions, such as muscular rest and digestive inactivity. The basal metabolism is a rather constant figure for the normal individual at rest and is, as shown by Lusk and his associates, chiefly dependent on the surface of the body. It has been shown by Peabody, Meyer, and Du Bois (21) that the metabolism in patients with compensated heart and cardiovascular lesions is within the normal limits. In patients with uncompensated heart lesions, particularly in patients suffering from dyspnea, however, the metabolism was found materially increased, in some cases about 50 per cent. An increase in the metabolism necessarily means

<sup>7</sup> Barcroft (19), p. 177.

an increase in the consumption of oxygen. This, however, is not necessarily followed by an increase in the deoxidation of the oxyhemoglobin. That depends upon the output of the heart, which may or may not be changed. Lindhard (9), for instance, has shown that the increased consumption of oxygen during exercise only partially shows itself in the decreased oxygen in the venous blood; the increased blood flow compensates for a great part of the increased consumption. Lunds-gaard (15) has shown that an increased oxygen consumption on account of exercise in two clinically compensated patients with heart block only to a very small degree could be compensated for by increasing blood flow. The reason for this was that it was impossible for the heart to increase the pulse rate and probably very difficult to increase the volume output per beat, which even in rest was about 150 cc. It is, therefore, probable that an increase in metabolism in a patient with a weak heart will increase the deoxidation of the venous blood, particularly in the venous blood coming from the heart muscle and the respiratory muscles. An increase in temperature may have a similar effect.

(3) About the only figures available on this point are those of Stewart (16). He has calculated from measurements of the heat given off by the hands and feet the rate of local blood flow and found that the flow in the right hand or foot is approximately equal to that in the left. Significant differences were encountered only when there was definite local cause, as aneurysms, diabetic gangrene, local edema, etc. It will probably be possible to throw light upon this question by simultaneously drawing blood from different veins and under different conditions, as tried in a number of cases by Means and Newburgh (11). Investigations on the local blood flow by Hewlett (22), using a modified Brodie method, have shown that there is a considerable temperature interval where the blood flow remains approximately constant when the surrounding temperature is changed.

The influence of the hemoglobin percentage, of the output from the heart, and of a possible unsaturation of the blood in the lungs is shown in diagram form in Text-fig. 2. The first double column represents the conditions in a person with normal hemoglobin, normal blood flow, and total saturation of the arterial blood. The next



**TEXT-FIG. 2.** Diagrams showing how the hemoglobin percentage, the output from the heart, and the saturation of the arterial blood influence the oxygen in the venous blood.

shows the findings in a patient with very low total oxygen capacity<sup>8</sup> but with normal circulation and full saturation in the lungs. A comparison of these two sets of figures shows the impossibility of using the zero as the base-line. The differences in hemoglobin must be accounted for. That is what we have done by using the term oxygen unsaturation, which is represented by the black area. The next two pairs of columns show how the extent of oxygen unsaturation can be affected in the same way by different causes. The third column represents the condition in a heart case (No. 14, Table II) when the total oxygen capacity was a little above normal; the saturation in the lungs was supposed to be normal. The cause of the increased oxygen unsaturation in this case was a slow circulation. The fourth column represents a hypothetical case with the same degree of oxygen unsaturation of the venous blood. However, the cause is ascribed to a considerable extent of unsaturation of the arterial blood. These two examples show the possible inaccuracy of using the word oxygen consumption in the same sense as oxygen unsaturation.

*Oxygen Unsaturation Compared with Oxygen Consumption  
(Blood Flow).*

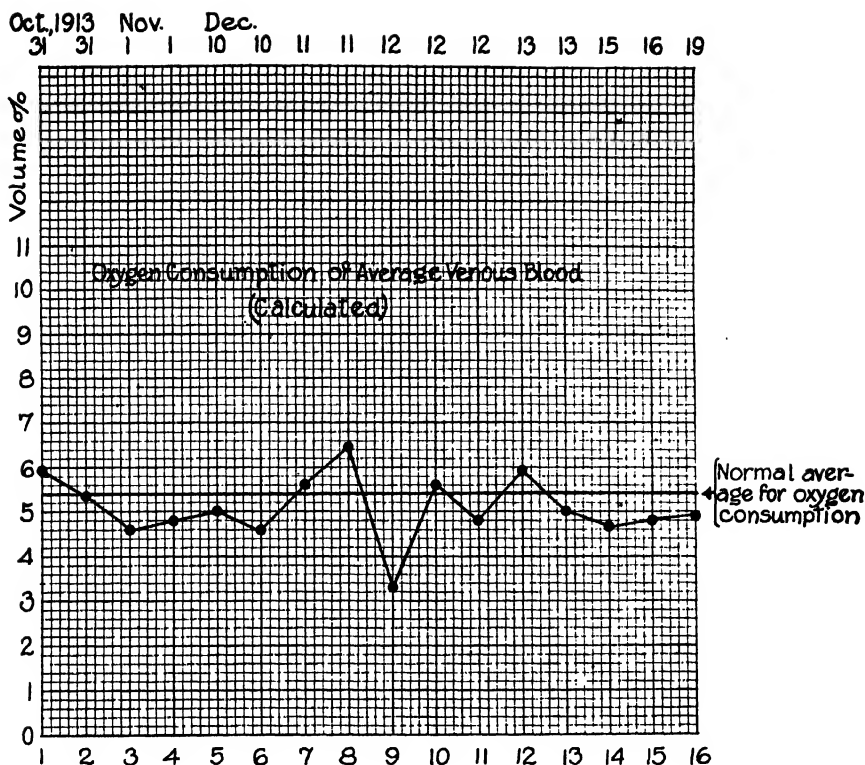
It is of interest for the interpretation of our results that the minute volume (output per minute from the heart) was determined 4 years ago in the Medical Clinic of the University of Copenhagen<sup>9</sup> on the same subject (the writer) on whom twenty determinations of the oxygen unsaturation of the venous blood have been done (Text-figs. 1 and 4).<sup>10</sup> From the figures indicating the values for the minute volume we can calculate the oxygen consumption of the average venous blood.<sup>11</sup>

<sup>8</sup> Compare Morawitz and Röhmer's observations (23).

<sup>9</sup> Lundsgaard (14), p. 397.

<sup>10</sup> See Table I, Paper I (1).

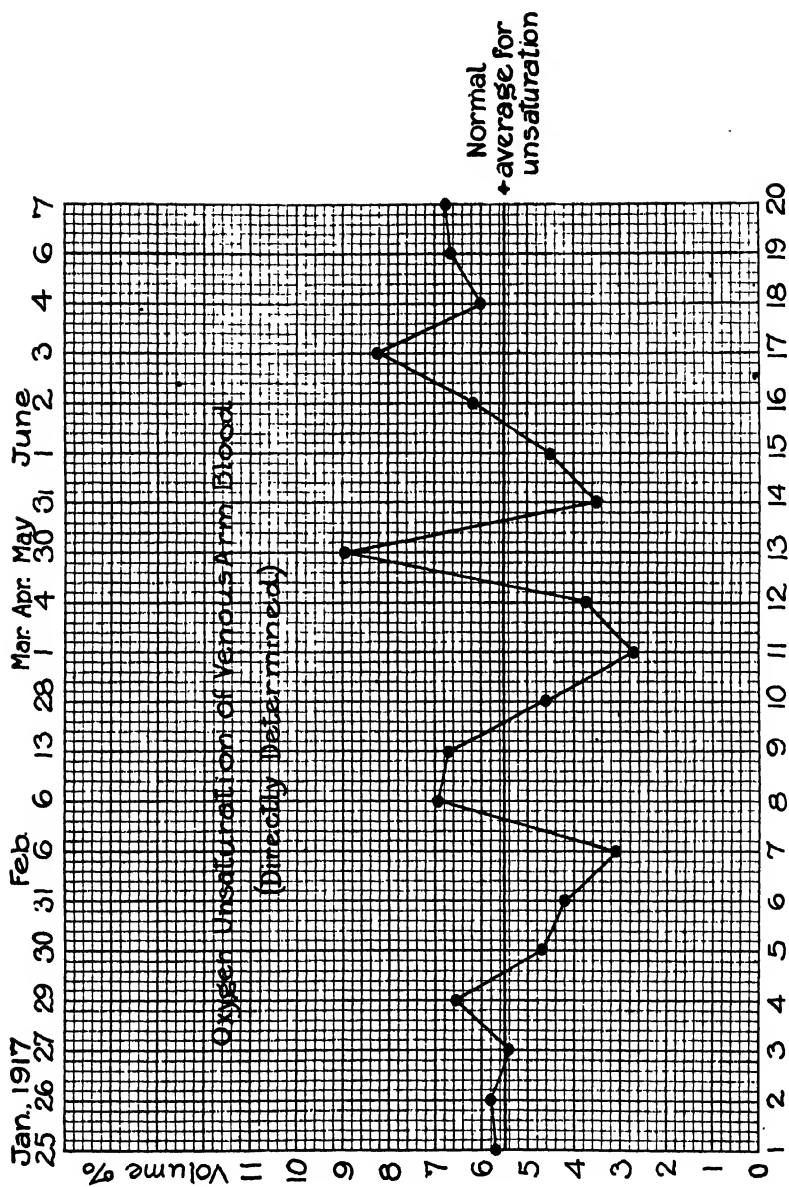
<sup>11</sup> The details of Krogh and Lindhard's method can be seen in the papers quoted in the bibliography, particularly in 8, 10, 13, and 14. The principles are the following: The subject is connected with an easily movable spirometer which contains a mixture of air and nitrous oxide. He mixes his lung air with the air in the spirometer during three to five respirations. He then stops breathing for a few seconds. Before and after the apneic period a sample of alveolar air is



TEXT-FIG. 3. Diagram showing the oxygen consumption of a normal man calculated from sixteen determinations of the minute volume of the heart by Krogh and Lindhard's nitrous oxide method.<sup>12</sup>

drawn and analyzed. On account of absorption by the blood flowing through the lung capillaries the second sample will contain a smaller amount of nitrous oxide and of oxygen than the first. The percentage in the difference between the two samples multiplied by the volume of air in the lungs will indicate the amount of blood flowing through the lungs during the period between the two samples. The output of blood per minute from the heart (the minute volume) can then be calculated. Knowing the quantity of oxygen taken up by the blood during the same period we can calculate the amount of oxygen taken up by the body per 100 cc. of blood. When a correction is introduced for a possible abnormality in the percentage of hemoglobin this amount will indicate the oxygen consumption by the average blood. An extensive critical study of Krogh and Lindhard's method is published by Sonne (24). He expresses doubt about the reliability of the method on account of possible imperfect mixture of the lung air, not only in patients but in normal individuals as well. Krogh and Lindhard (25) have later on admitted that the difficulties shown by Sonne may exist.

<sup>12</sup> See Lundsgaard (14); p. 397, where the data for the blood flow can be obtained.



TEXT-FIG. 4. Diagram showing the oxygen unsaturation of the venous arm blood from the same normal subject<sup>4</sup> as in Text-fig. 3 (see also Text-fig. 1).



Sixteen determinations of the blood flow were made at that time. The calculated figures for the oxygen consumption in these sixteen experiments are given in Text-fig. 3. The average oxygen consumption for a considerable number of blood flow experiments on normal people (5.4 volume per cent) is indicated.

In Text-fig. 4 are given the figures for the oxygen unsaturation of the venous arm blood of the same subject (the writer) as reported in the first paper of this series.<sup>10</sup> The average line in this diagram (5.5 volume per cent) is from thirty-eight determinations of the oxygen unsaturation on eleven normal people reported in Paper I and shown in Text-fig. 1 in this paper. It will be seen how closely the values for the oxygen unsaturation determined by Van Slyke's method agree with the values for the oxygen consumption calculated from the determinations of the blood flow with Krogh and Lindhard's method. The average figures for normal individuals are the same (5.5 and 5.4 volume per cent). The variations for the two series on the same person agree rather closely. The variations in the oxygen unsaturation are more extensive than the variations in the values for the average consumption; *i.e.*, for the blood flow. This is probably due to variations in the local blood flow in the arm from which the blood is drawn. It is worth mentioning that the subject on whom the determinations were done has a very labile circulatory system (respiratory arrhythmia, changeable pulse, dermatographia).<sup>13</sup> The significance of this agreement is that the amount of oxygen lost by 100 cc. of blood in passing through the forearm is approximately the same as the average loss in passing through the other body tissues. In view, nevertheless, of the undoubted possibility that the disturbing factors discussed may influence the unsaturation, we are not justified at present in interpreting unsaturation figures in terms of minute volume of the heart. What we believe we can do, is to fix the limits of the oxygen unsatura-

<sup>13</sup> The conception that the extensive variations in the oxygen unsaturation in this particular case are principally due to vasomotor changes, is supported by an observation (Cohn and Lundsgaard, personal communication) on the relation between the brachial blood pressure and the blood pressure in the arteries of the finger. The tension in the digital arteries was found more variable than in other normal subjects and more variable than the blood pressure nearer the center.

tion in subjects with normal circulation and normal lungs and study the variations observed in carefully controlled patients with symptoms of abnormal circulation and in patients with respiratory abnormalities. From the data thus obtained we may empirically standardize the figures for the oxygen unsaturation, learn the pathological conditions that affect it, and thus add it to the armamentarium that assists the clinician in accurate diagnosis. The empirical evolution of blood pressure measurement, made possible by accumulation of many determinations on clinically controlled patients, has shown how a quantitatively measurable factor, even though imperfectly explained physiologically, may prove to be of value in clinical medicine.

#### SUMMARY.

1. Thirty-one determinations of the total oxygen-combining power and the oxygen in the venous blood from vena mediana cubiti of sixteen resting patients are reported.
2. The difference between the total oxygen capacity of the hemoglobin and the oxygen in the venous blood, the oxygen unsaturation, is calculated.
3. In twelve patients with compensated heart lesions the unsaturation was found within normal limits, between 2.5 and 8 volume per cent.
4. In four patients with uncompensated heart disease the values for the unsaturation were all above the normal limit, from 9.7 to 15.2 volume per cent.
5. A general discussion of the problem of interpreting the results is given.
6. A comparison is drawn between the oxygen consumption calculated from direct determination of the blood flow on a normal subject (the writer) and the oxygen unsaturation determined 4 years later on the same subject. A close agreement between the two series of values exists.

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## STUDIES OF OXYGEN IN THE VENOUS BLOOD.

### III. DETERMINATIONS ON FIVE PATIENTS WITH COMPENSATED CIRCULATORY DISTURBANCES.

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#### INTRODUCTION.

In two previous papers on the same subject (1, 2) the technique of drawing blood from the arm vein and a series of determinations on twelve normal individuals and sixteen patients with circulatory disturbances have been reported.

In the discussion of the interpretation of the results, it was pointed out that variations in the oxygen unsaturation of the blood from an arm vein might be caused by several factors other than the output from the heart, which we were able to control or eliminate only to a limited degree. These factors were: (1) the degree of oxygen saturation of the arterial blood; (2) the oxygen consumed by the metabolism of the part of the body drained by the vein tapped; (3) changes in the local blood flow on account of vasodilatations or constrictions either in the organ (the arm) from which the blood is drawn or in other parts.

In animal experiments the influence of these factors might be studied separately. In clinical medicine, however, we have to deal with a given condition where several factors usually act together. We are, therefore, compelled to compare our findings with the clinical observations and postpone the conclusions until sufficiently extensive material has been collected.

The purpose of these investigations is to study the variations in the oxygen unsaturation of the venous blood in patients with symptoms of circulatory disturbances; that is, in patients in whom it is of importance to find out whether the blood flow is changed or not.

The preliminary determinations reported in Paper II gave promising results. Continuous determinations on patients over a longer period have therefore been taken up. Ten patients have been studied, five with compensated<sup>1</sup> and five with uncompensated circulatory disturbances. This paper is a report of the findings in the compensated group.

Before reporting the clinical notes and the figures for the oxygen unsaturation in these five patients, one should recall that the limits for the oxygen unsaturation in normal individuals were 9 and 2.5 volume per cent. However, values between 8 and 9 volume per cent were found in only four determinations and under special circumstances—a few seconds after awakening from a night's sleep, a condition which, as a rule, we shall be unable to reproduce in patients. Considering that all the values for the oxygen unsaturation in twelve patients with compensated circulatory disturbances (2) fell between 8 and 2.5 volume per cent, it seems correct, at least for the time being, to consider 8 volume per cent the upper normal limit. The lower limit is 2.5 volume per cent, the average 5.5. In the diagrams of the oxygen unsaturation lines have been drawn to indicate the normal limits.

#### CASES.

*Case 1 (Table I, Text-Fig. 1).—V. G., male, gardner; age 58 years.*

*Diagnosis.*—Aortic insufficiency; arteriosclerosis; hypertension.

*Previous History, Symptoms, etc.*—Dyspnea after slight exertion, for 1 year. He has never had syphilis or rheumatic fever, but for years he has suffered from stiffness of the back and has now and then had slight joint pains.

*Physical Examination.*—No cyanosis; no swelling of superficial glands; lungs clear; heart dullness increased considerably to the left and downwards; x-ray shows increase to the left and downwards; no thrill. At the fourth rib on the left side is heard a distinct diastolic murmur replacing the second sound, also heard at apex; liver not felt; no ascites; no edema. In the urine was found a trace of albumin; no casts. Blood pressure 150–170. Wassermann reaction negative. Temperature normal.

He was admitted to the hospital for diagnosis, confined to bed for the first 3 days, and after that time allowed to get out of bed. No special treatment. He felt better when discharged; dyspnea less; physical signs unchanged.

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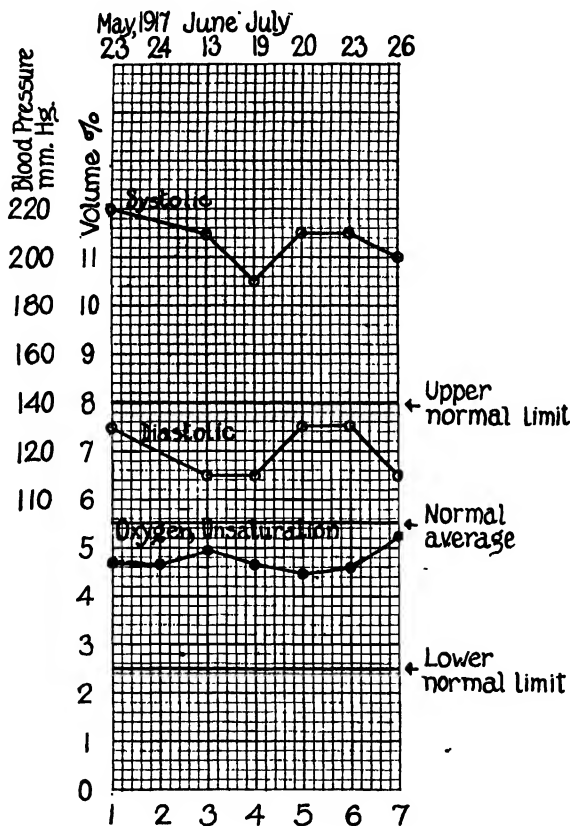
<sup>1</sup> Patient 4 in the present paper had symptoms of heart failure. During the course of the disease the symptoms were interpreted as not circulatory.

TABLE I.  
*Case 1. Aortic Insufficiency; Arteriosclerosis; Hypertension; Hypertrophy of Left Side of Heart.*

Determination No.		Bleeding.			Condition.*	Oxygen content of venous blood.								Hemoglobin (g. m. c. method).	Calculated oxygen capacity (g.)	Oxygen unsaturation (g. %).	Pulse.	Respirations.
		Date.	Hour.	Arm.		Sample 1.		Sample 2.		Average (v).								
						Hour.	Result.	Hour.	Result.									
		1917					vol. per cent		vol. per cent		vol. per cent		vol. per cent		vol. per cent			
1		May 23	1.00 p.m.	Left.	$\frac{1}{2}$ hr.	2.30	13.90	2.50	13.82	100	18.50	100	18.50	4.64	72	24		
2		" 24	1.00 "	Right.	$\frac{1}{2}$ "	3.00	14.29	3.10	13.64		(18.50)		(18.50)	4.53	76	20		
3		June 13	11.50 a.m.	"	$\frac{1}{2}$ "	12.20	13.55	12.50	13.46	100	18.50	100	18.50	4.99	66	24		
4		July 19	11.00 "	Left.	1 $\frac{1}{2}$ hrs.	11.20	14.01	12.00	14.89	103	19.06	103	19.06	4.61	22	22		
5		" 20	11.50 "	"	18 "	1.10	14.68	1.30	14.68	103	19.06	103	19.06	4.48	66	22		
6		" 23	9.30 "	Left and right.	12 "	10.20	14.60	10.50	14.32	103	19.06	103	19.06	4.60	66	22		
7		" 26	9.40 "	Right.	$\frac{1}{2}$ hr.	10.20	13.82	10.40	13.62	103	19.06	103	19.06	5.34	72	24		

\* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

This patient, who suffered from a valvular heart lesion which is fully compensated at rest, shows almost constant values for the oxygen unsaturation from day to day. Furthermore the values follow the normal average. He was a patient with a quiet temper, with very slowly reacting vasomotors, and with a steady, regular pulse.



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of aortic insufficiency; arteriosclerosis; hypertension.

*Case 2 (Table II, Text-Fig. 2).—T., male, steward; age 52 years.*

*Diagnosis.*—Hypertension arterialis; slight arteriosclerosis; chronic alcoholism; cirrhosis of the liver (?); lues.

*Previous History, Symptoms, etc.*—Shortness of breath on exertion, for 4 years.

*Physical Examination.*—Rather fat man; lungs clear; no cyanosis; no prominent



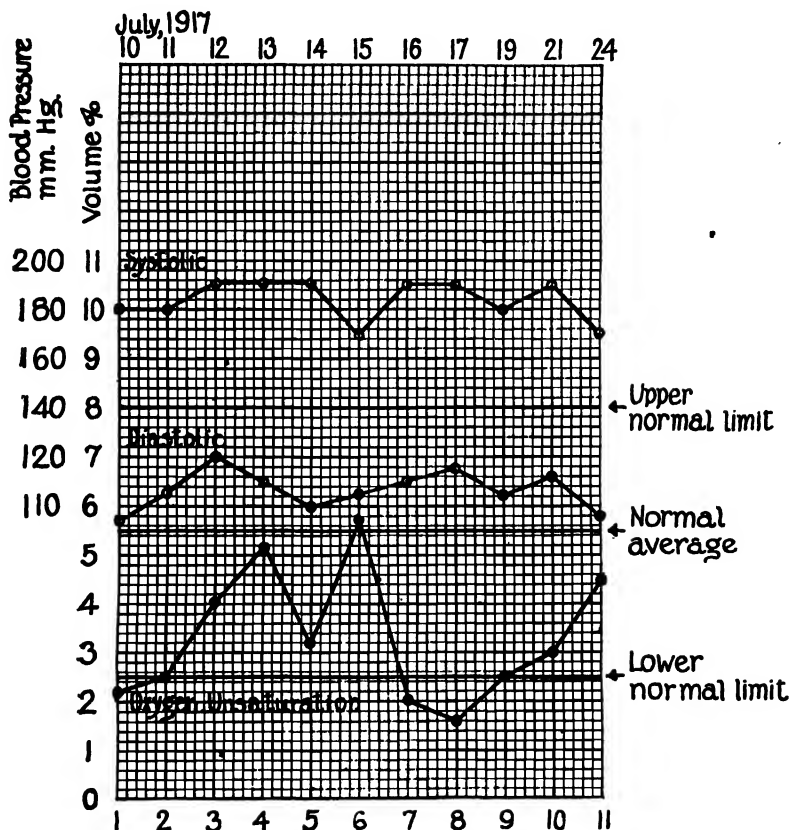
TABLE II.

Case 2. Arterial Hypertensions; Slight Arteriosclerosis; Cirrhosis of Liver; Luas; Chronic Alcoholism.

Determination No.	Bleeding.				* Oxygen content of venous blood.							Hemoglobin (Palmers method).	Calculated oxygen capacity (a).	Oxygen unsaturation (v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).							
					Hour.	Result.	Hour.	Result.								
	1917				vol. per cent		vol. per cent	vol. per cent		vol. per cent	vol. per cent	vol. per cent				
1	July 10	9.30 a.m.	Left.	In bed.	10.30	17.02	10.50	16.80	16.91	103	19.06	2.16	73	20		
2	" 11	9.30 "	"	"	4.10	16.62	4.40	16.62	16.62	103	19.06	2.44	84	24		
3	" 12	9.30 "	Right.	"	10.10	15.05	10.50	15.05	15.05	103	19.06	4.01	87	20		
4	" 13	9.30 "	"	"	11.10	13.83	3.40	13.99	13.91	103	19.06	5.15	92	28		
5	" 14	9.30 "	"	12 hrs.	2.10	14.60	2.20	14.80	14.70	97*	17.92	3.22	92	22		
6	" 15	12.20 p.m.	"	12 "	12.40	12.07	12.55	12.81	12.44	98*	18.13	5.69	91	22		
7	" 16	10.30 a.m.	"	12 "	10.40	16.85	10.53	16.85	16.85	102	18.82	1.97	90	24		
8	" 17	9.20 "	"	12 "	10.20	17.50	11.00	17.40	17.45	103	19.06	1.51	92	28		
9	" 19	9.30 "	Left.	12 "	10.00	15.97	10.20	15.97	15.97	100	18.50	2.53	98	22		
10	" 21	9.30 "	"	12 "	9.40	15.82	10.10	15.18	15.50	100	18.50	3.00	96	24		
11	" 24	9.30 "	Right.	1 hr.	10.00	13.25	10.15	13.25	13.25	96	17.74	4.49		26		

\* Checked three times.

veins; heart covered by the lungs; limits uncertain at percussion; x-ray shows the heart moderately enlarged to the left; no murmurs; aortic second sound high pitched and accentuated. The abdomen is fat with prominent veins on both sides from the inguinal region to the axilla. Liver not felt; area of dullness over spleen not distinctly increased; no jaundice; no ascites; no edema; the urine con-

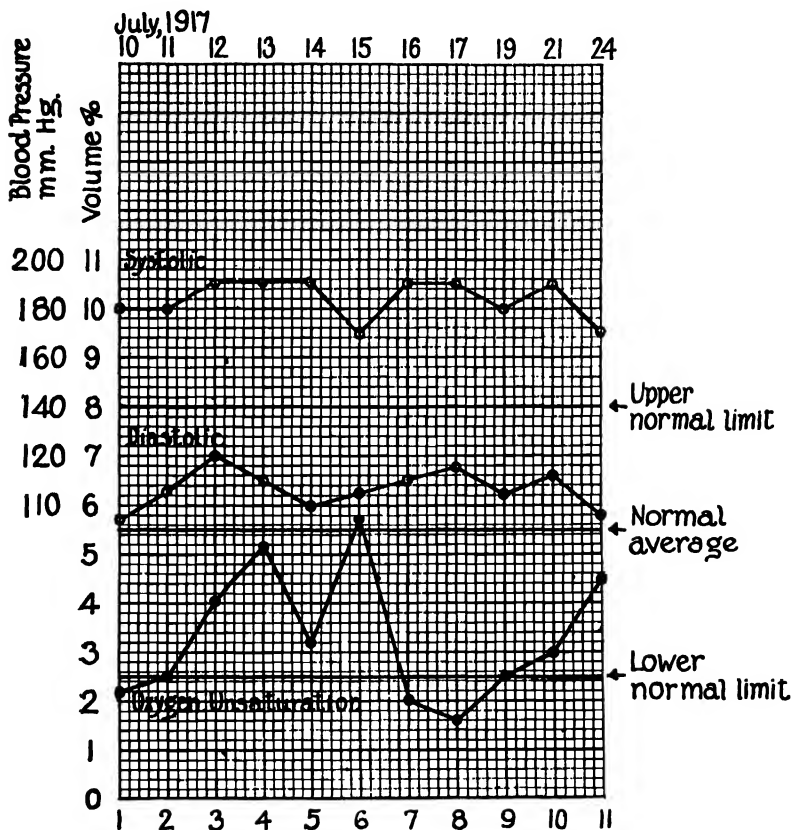


TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of arterial hypertension; slight arteriosclerosis; cirrhosis of liver; lues.

tains a faint trace of albumin; no casts; blood urea not increased. Wassermann reaction positive. Temperature normal.

*Treatment.*—Confined to bed; restricted fluid and calories; no medication apart from antisyphilitic treatment, which was started after the determinations of the oxygen were finished.

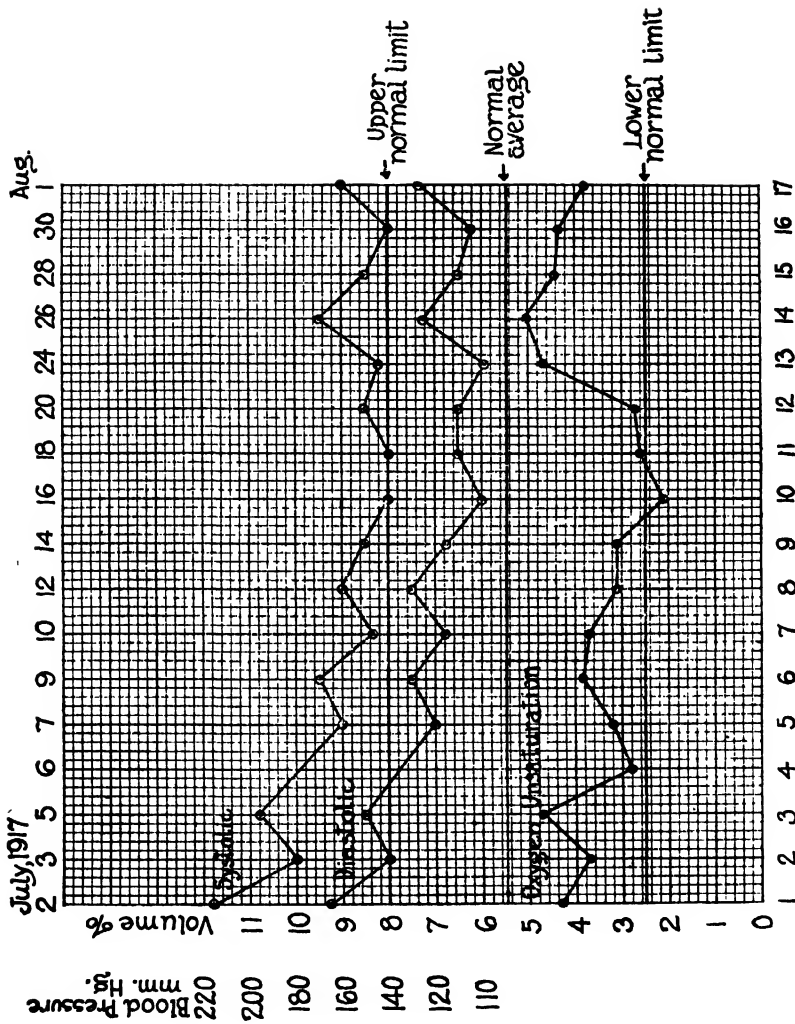
veins; heart covered by the lungs; limits uncertain at percussion; x-ray shows the heart moderately enlarged to the left; no murmurs; aortic second sound high pitched and accentuated. The abdomen is fat with prominent veins on both sides from the inguinal region to the axilla. Liver not felt; area of dullness over spleen not distinctly increased; no jaundice; no ascites; no edema; the urine con-



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of arterial hypertension; slight arteriosclerosis; cirrhosis of liver; lues.

tains a faint trace of albumin; no casts; blood urea not increased. Wassermann reaction positive. Temperature normal.

*Treatment.*—Confined to bed; restricted fluid and calories; no medication apart from antisyphilitic treatment, which was started after the determinations of the oxygen were finished.



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of arteriosclerosis; hypertension; lues; aneurysm; aortic insufficiency.

TABLE III.

Case 3. *Arteriosclerosis; Hypertension; Lues; Incipient Aneurysm of the Aorta.*

Determination No.	Bleeding.				Oxygen content of venous blood.						Hemoglobin (g. per cent)	Calculated oxygen capacity (g.)	Oxygen unsaturation (g.)	Pulse.	Respirations.
	Date.	Ho. r.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v.).						
					Hour.	Result.	Hour.	Result.							
1	1917	July 2	2.00 p.m.	Left.	1 hr.	2.10	17.14	2.30	17.32	17.23	116	21.48	4.25	108	20
2	"	3	9.30 a.m.	"	In bed.	10.00	17.63	10.30	17.86	17.75	116	21.48	3.73	80	14
3	"	5	11.30 "	"	"	12.00	16.46	1.00	17.02	16.74	116	21.48	4.74	88	20
4	"	6	9.50 "	"	"	4.40	18.64	5.20	18.64	18.64	116	21.48	2.84	88	20
5	"	7	9.20 "	"	"	9.40	18.24	10.20	18.44	18.34	116	21.48	3.22	94	24
6	"	9	11.00 "	"	"	11.50	18.44	12.00	18.22	18.33	120	22.20	3.87	80	20
7	"	10	4.30 p.m.	"	"	5.30	15.56	6.00	18.54	18.55	120	22.20	3.65	80	22
8	"	12	9.20 a.m.	"	"	11.00	18.95	11.20	20.05	19.50	122	22.54	3.04	80	22
9	"	14	9.40 "	"	½ hr.	11.00	17.45	•		17.45	111*	20.54	3.09	81	20
10	"	16	9.30 "	"	¾	10.00	18.44	10.30	18.50	18.47	111	20.54	2.07	86	24
11	"	18	9.50 "	"	1	11.30	16.86	11.50	17.30	17.08	106	19.62	2.54	84	24
12	"	20	9.30 "	Right.	¾	9.50	17.03	10.20	17.24	17.14	107	19.80	2.66		
13	"	24	9.30 "	Left.	¾	11.00	14.53	11.30	14.53	14.53	104	19.25	4.72	88	26
14	"	26	9.30 "	"	¾	10.50	14.22			14.22	104	19.25	5.03	96	20
15	"	28	9.30 "	Right.	1	9.50	14.84			14.84	104	19.25	4.41	72	20
16	"	30	9.30 "	Left.	¾	9.50	13.92	10.30	14.34	14.13	100	18.50	4.37	80	24
17	Aug.	1	9.30 "	Right.	¾	9.50	14.88	10.30	14.54	14.71	100	18.50	3.79	94	24

\* The determination of the hemoglobin was repeated three times on the blood in which the oxygen was determined. Furthermore, a sample was drawn from the ear, which gave the same result. The standard was also tested.

This patient was a moderately advanced case of the cardiovascular type. Besides he probably had some cirrhosis of the liver. His circulation was fully compensated. The oxygen unsaturation shows less constancy than in Case 1. However, the variations extend practically only over the half of the area which is covered by the values for the normal individuals. The variations are smaller than in the normal person and the curve as a whole is more uniform.

*Case 3 (Table III, Text-Fig. 3).—L., male, musician; age 35 years.*

*Diagnosis.*—Arteriosclerosis; hypertension; lues; incipient aneurysm of the aorta.

*Previous History, Symptoms, etc.*—Headache; shortness of breath on exertion; contracted syphilis 6 years ago; treated regularly.

*Physical Examination.*—The site of the heart is normal to percussion and x-ray; no murmurs; both aortic and pulmonic second sounds very distinct; aortic particularly high pitched; heart action regular; electrocardiogram normal. The x-ray picture shows a slight distention of the left side of the upper part of the descending aorta. Wassermann test doubtful three times. Lungs clear; no cyanosis, distended veins, or edema; liver not felt; radial and temporal arteries tortuous and very hard; urine negative for albumin. For the blood pressure see Text-fig. 3. Temperature normal.

*Treatment.*—Confined to bed for the first 12 days; salt-free diet; restricted calories and fluid.

During his stay in bed his hemoglobin increased from 116 to 122. The 2nd day he was out of bed 3 hours, his hemoglobin dropped suddenly to 111 (verified several times on two different samples of blood) and then slowly down to 100 per cent. No explanation for this could be found in his condition or treatment.

We have here a patient with a pronounced premature arteriosclerosis, probably on syphilitic basis. Besides that he had a beginning aneurysm which could be detected only by x-ray. His shortness of breath on exertion is probably due entirely to this high blood pressure. The values for the oxygen unsaturation in seventeen determinations are rather constant and, like the values in Case 2, occupy the space below the normal average, one a trifle below the normal lower limit.

*Case 4 (Table IV, Text-Fig. 4).—J. A., male, porter; age 49 years.*

*Diagnosis.*—Cirrhosis of the liver; nephritis; urea retention; uremia (?); anemia.

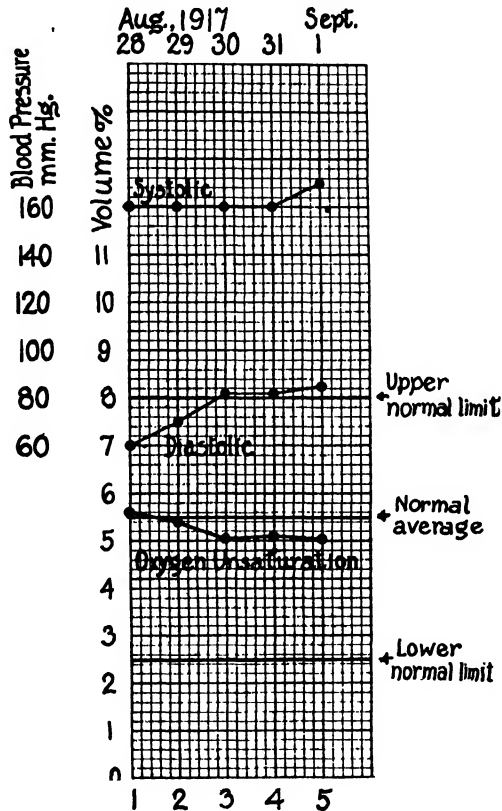
*Previous History, Symptoms, etc.*—Edema of legs for 3 to 4 months; has used much alcohol; has never had any sickness before.

*Physical Examination.*—Dullness from the right border of sternum to 2 cm. below the nipple. X-ray shows that the heart is in a transverse position (on account of his ascites); the size and form not changed. All over the precordia a

TABLE IV.  
Case 4. *Nephritis; Uremia; Cirrhosis of Liver; Anemia.*

Determination No.	Bleeding.				Oxygen content of venous blood.						Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).						
					Hour.	Result.	Hour.	Result.							
	1917					vol. per cent			vol. per cent	per cent	vol. per cent	vol. per cent			
1	Aug. 28	9.30 a.m.	Right.	In bed.	9.50	7.80			7.80	72	13.32	5.52		79	22
2	" 29	9.30 "	"	"	9.50	7.90	10.10	7.90	7.90	72	13.32	5.42		78	22
3	" 30	4.00 p.m.	"	"	4.10	8.16	4.30	8.36	8.26	(72)	(13.32)	5.06		94	26
4	" 31	10.00 a.m.	"	"	10.20	7.85	10.40	7.85	7.85	70	12.94	5.09		78	28
5	Sept. 1	6.50 "	"	"	8.20	8.28	8.40	8.28	8.28	72	13.32	5.04		96	24

soft systolic murmur;<sup>2</sup> aortic and pulmonic second sounds normal; pulse regular; electrocardiogram normal; the day of the first determination some moist râles in lungs; since then clear; no cyanosis; veins are not distended; marked ascites; no jaundice; liver and spleen not felt, but the area of dullness over the spleen is distinctly increased; uremic odor of breath; sleepy; heavy edema of lower extremi-



TEXT-FIG. 4. Oxygen unsaturation of venous arm blood. Case of cirrhosis of liver; nephritis; uremia; anemia.

ties extending up over the lower part of abdomen; diuresis small. Urine contains albumin; granular and hyaline casts; urea in blood somewhat increased (50 mg. of urea per 100 cc. of blood). Condition stationary during the time of bleeding.

*Treatment.*—Confined to bed; 800 cc. of milk per day. Diuresis very small (200 to 400 cc.). For the blood pressure see Text-fig. 4.

<sup>2</sup> He was admitted as a case of uncompensated heart failure.



The diagnosis in this case was confirmed by an autopsy which showed a typical liver cirrhosis of Laennec's type, a big hard spleen, parenchymatous, subchronic nephritis; heart and lungs were normal; no sclerosis of aorta. The results on this patient show the importance of using the oxygen unsaturation, not the direct value for the oxygen in the venous blood, on account of the anemic condition. A consideration of the whole clinical picture made it obvious that his ascites, edema, and low diuresis were not due to incompenated circulation. It is, therefore, interesting, to find normal values for the oxygen unsaturation. The values are not only within the normal limits, but they are very constant and follow absolutely the line for the normal average. The day when the first determination was made quite a number of moist râles were heard at the base of the left lung. At the time of the following determinations the lungs were clear. However, no differences were observed in the figures for the oxygen unsaturation.<sup>3</sup> He was very quiet all the time, in fact his mind was not quite clear, particularly at the time of the last determinations. The temperature was normal. He died 5 days after the last determination.

*Case 5 (Table V, Text-Fig. 5).—G., male, teacher; age 38 years.*

*Diagnosis.*—Mitral insufficiency; cardiac palpitations; auricular fibrillation.

*Previous History, Symptoms, etc.*—Palpitation; fatigue on exertion, for 4 to 6 years; no rheumatic fever; no syphilis.

*Physical Examination.*—On admission moist râles at the base of the left lung; otherwise lungs are clear; no cyanosis or swelling of superficial vein; dyspnea only on rather considerable exertion; dullness distinctly increased to the left and in direction of the left axilla; at apex distinct systolic murmur replacing almost entirely the first sound; no diastolic murmur; pulse rate moderately rapid (100); electrocardiogram and pulse show auricular fibrillation; liver not felt, not tender; no edema. Temperature normal. From April 19 to 30 he had 0.5 gm. of digipuratum a day. He did not show any clinical signs of incompensations. The râles in his lungs disappeared and the lungs were clear at the time of the determinations; temperature normal.

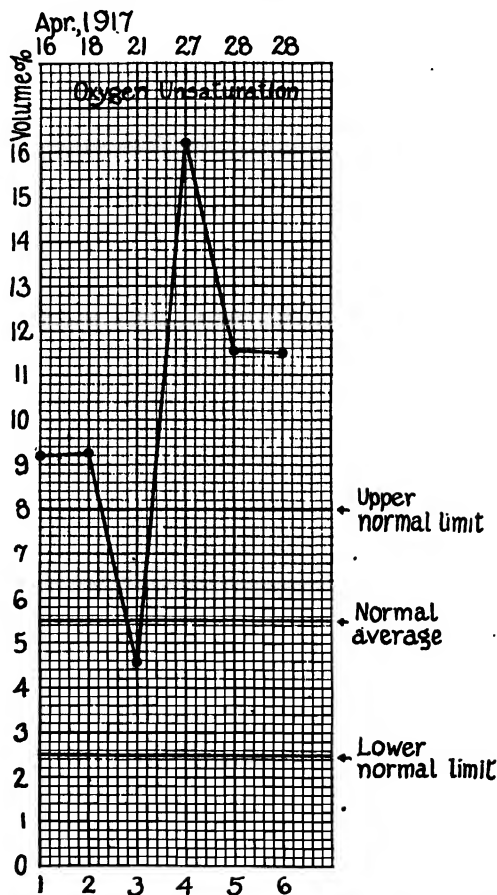
The results show two very important traits. In the first place, the values for the oxygen unsaturation vary to an extent not seen before, from 4.5 to 16.2 volume per cent. In the second place, he is the first patient with a clinically compensated heart lesion in which there have been found values for the oxygen unsaturation outside the normal limits, 8 and 2.5 volume per cent. The results on this patient are in striking contrast to those found in the other four patients.

<sup>3</sup> See the discussion in Paper II (2) about the saturation of the arterial blood.

TABLE V.  
Case 5. *Mitral Insufficiency; Cardiac Palpitations; Auricular Fibrillation.*

Determination No.	Bleeding.			Condition.	Oxygen content of venous blood.						Hemoglobin (palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Pulse.		Respirations.
	Date.	Hour.	Arm.		Sample 1.		Sample 2.		Average (v).	Apex.				Radical.		
					Hour.	Result.	Hour.	Result.								
															vol. per cent	
1	1917	Apr. 16	3.00 p.m.	Right.	In bed.	3.40	10.00	3.55	10.08	10.04	104	19.24	9.20	84	20	
2	"	"	11.30 a.m.	"	"	12.10	9.91	12.30	10.25	10.08	104	19.30	9.22	64	20	
3	"	"	11.40 "	"	2 hrs.	2.40	15.71	2.55	15.75	15.73	118	21.28	4.55	87	19	
4	"	"	3.10 p.m.	"	1 hr.	3.30	5.81	3.50	6.25	6.03	120	22.20	16.17	74	64	
5	"	"	11.00 a.m.	Left.	3 hrs	12.00	9.01	12.30	9.27	9.14	114	20.70	11.56	86	22	
6	"	"	11.15 "	"	3 "	12.15	9.27	1.30	9.11	9.18	(114)	(20.70)	11.52	81	21	

The most conspicuous clinical difference between him and the other four patients is the heart rhythm. It is difficult to find other cause for the change in the oxygen unsaturation than variations in the output from the heart. The lungs were clear, there was no evidence of any



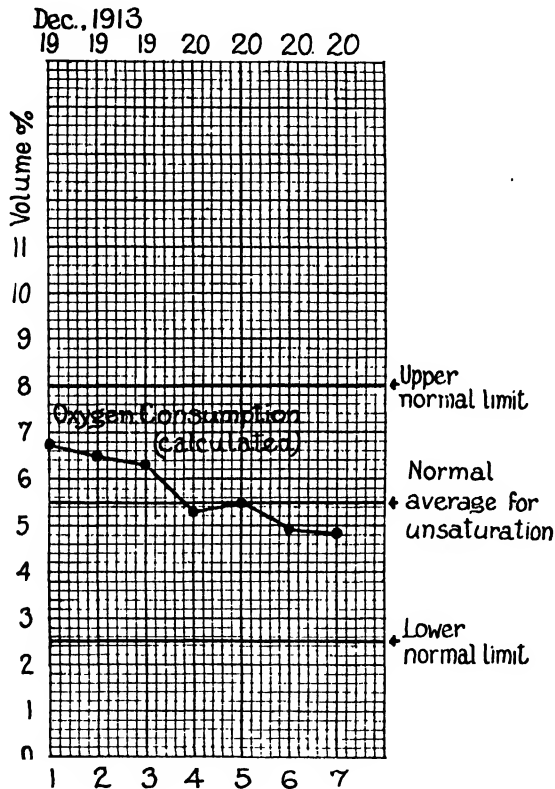
TEXT-FIG. 5. Oxygen unsaturation of venous arm blood. Case of mitral insufficiency and auricular fibrillation.

changes in the metabolism, and it seems unlikely that undetected vaso-motor changes could account for the fluctuations shown in the chart.

#### DISCUSSION.

A previous investigation of the minute volume in heart patients by the writer (3) gave results which agree closely with those found here.

It was found that patients with valvular disease combined with arrhythmia perpetua or with groups of extrasystoles may show (1) abnormally great variations in the output and (2) diminished minute volume without clinical signs of incompensation. It is possible to calculate the degree of oxygen consumption<sup>4</sup> in the patients referred

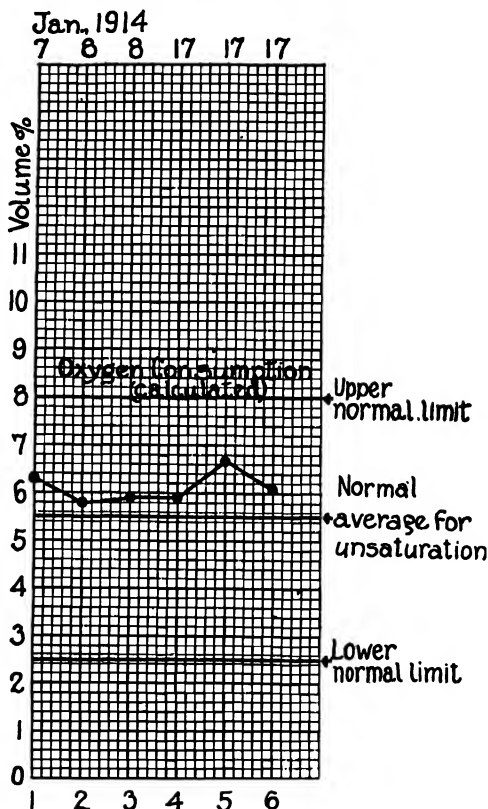


TEXT-FIG. 6. Case of compensated mitral and aortic insufficiency and mitral stenosis. Pulse regular.<sup>5</sup> Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

<sup>4</sup> The difference between oxygen unsaturation and oxygen consumption is defined in Papers I and II (1, 2). In Paper II is given a short description of Krogh and Lindhard's method.

<sup>5</sup> Case 1, Lundsgaard (3), p. 518.

to. Text-figs. 6 to 9 show the calculated values for the oxygen consumption in four patients where the minute volume was determined by Krogh and Lindhard's nitrous oxide method. Text-figs. 6, 7, and 8 represent patients suffering from compensated heart lesions. The

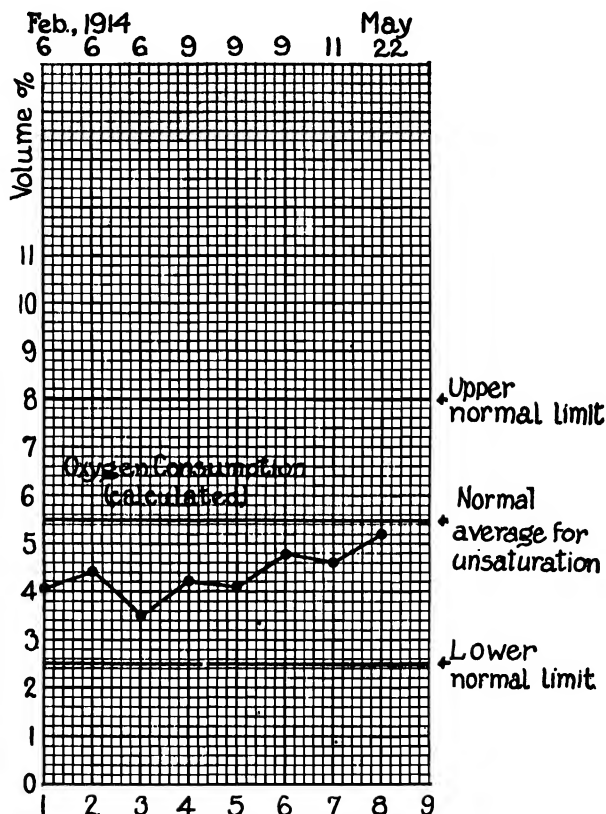


TEXT-FIG. 7. Case of compensated mitral stenosis and insufficiency. Pulse regular.<sup>6</sup> Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

pulse rate was regular in these cases. It will be seen that the figures agree closely with the directly determined oxygen unsaturation in the first four patients in this paper.

<sup>6</sup> Case 10, Lundsgaard (3), p. 544.

Text-fig. 9, on the other hand, represents a patient with clinically compensated mitral stenosis and auricular fibrillation. The calculated values for the oxygen consumption (1) are varying, (2) are above the normal line, and (3) agree closely with the directly determined

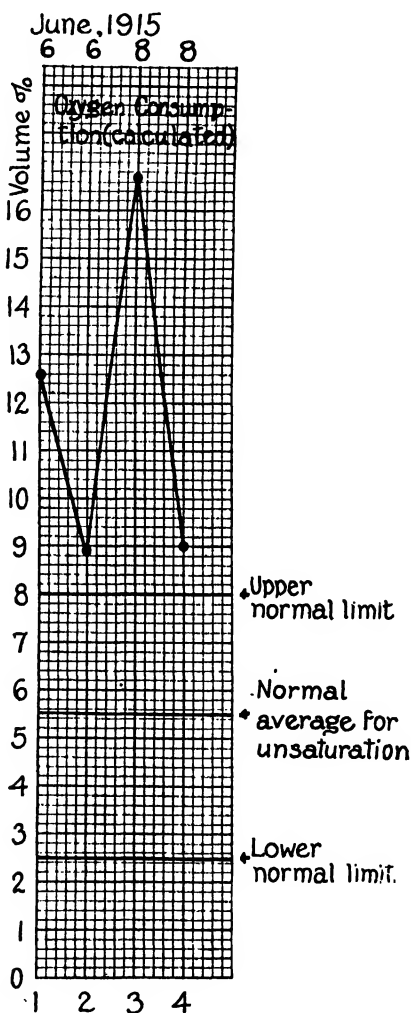


TEXT-FIG. 8. Case of compensated heart block.<sup>7</sup> Pulse regular. Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

values in Case 5 of this report. This seems to be sufficient proof that the variations in the oxygen saturation of the venous arm blood may in some instances express the variations in the output of the heart.

<sup>7</sup> Case 1, Lundsgaard (4), p. 488.

The values for the oxygen unsaturation in the first four patients are, as mentioned before, more uniform than the values in normal



TEXT-FIG. 9. Case of compensated mitral stenosis. Pulse irregular. Auricular fibrillation.<sup>8</sup> Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

<sup>8</sup> Case 7, Lundsgaard (3), p. 535.

people. The same thing could be seen in the determinations of the minute volume of the heart in normal people and in patients with compensated circulatory disturbances. The figures for the oxygen unsaturation calculated from the blood flow were more regular in patients with compensated valvular disease and regular heart action than in the two normal people. This may be interpreted as indicating that a regularly beating heart working against a certain burden will have a smaller margin of action than the normal heart. The burden probably acts on the heart like a heavy wheel on a machine, making its action steady.<sup>9</sup>

#### SUMMARY.

1. Forty-six determinations of the oxygen unsaturation, *i.e.*, the difference between the venous oxygen and the total oxygen capacity of the hemoglobin, have been done in five patients with compensated circulatory disturbances.

2. Values within normal limits and near or below the normal average were found in four patients with regular pulse.

3. In one patient with mitral insufficiency and arrhythmia perpetua extremely varying values were encountered. Six determinations were made; in one instance the value was within normal limits, in five above, and in one of those even higher than in most cases of uncompensated heart lesions.

4. A comparison is drawn between the directly determined oxygen unsaturation in these patients and the oxygen consumption calculated from previous experiments by the writer, where the blood flow was determined directly by the nitrous oxide method. A close parallelism is found.

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<sup>9</sup> It is worth mentioning that the normal person on whom twenty determinations of oxygen were done (Paper I and Text-fig. 1, Paper II) had a very unsteady circulation (unsteady pulse, respiratory arrhythmia, dermatographism). Great variations in the oxygen unsaturation were encountered.





## STUDIES OF OXYGEN IN THE VENOUS BLOOD.

### IV. DETERMINATIONS ON FIVE PATIENTS WITH INCOMPENSATED CIRCULATORY DISTURBANCES.

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(Received for publication, October 23, 1917.)

#### INTRODUCTION.

The technique of drawing and preserving the blood, the results on normal individuals, and a preliminary study of patients with circulatory disturbances have been reported in Papers I and II (1, 2) of this series.

In accordance with the viewpoints laid down in these two papers an investigation of the variations in the oxygen unsaturation<sup>1</sup> of the venous blood over a longer period in the same person was taken up. Ten patients suffering from different forms of circulatory disturbances have been studied. In Paper III (3) a report was made of 46 determinations on five patients in a compensated stage. The present paper is a report of 103 determinations on five patients with different heart lesions, all of whom, during a certain period, were distinctly clinically uncompensated. Repeated determinations have been made during the course of the disease, and the clinical symptoms which have bearing upon the compensation or uncompensation have been noted.

Short descriptions of the history, the physical examination at admission, and the treatment are given. The development of the disease can be followed from the notes on the charts. In the diagrams are given the curves of the oxygen unsaturation of the venous blood.

<sup>1</sup> The term oxygen unsaturation means the difference between the total oxygen-combining power of the hemoglobin and the oxygen in the venous blood. For further details see the discussion in the preceding papers, especially Paper II, Text-fig. 2.

The extreme normal limits and average normal are indicated on the diagrams for comparison. Marks showing the treatment with digitalis and the condition of the circulation (compensation or incompen-sation) are added. The clinical symptoms, which have been especially followed, are dyspnea, cyanosis, swelling of the superficial veins, enlargement of the liver, edema, diuresis, and body weight. The figures for the respiration indicate the rate just after the blood has been drawn. For that reason the rate of respiration is sometimes somewhat higher than would be the case if the patient had not been bled. This holds particularly true in the compensated stage.

The pulse has been counted during the bleeding. In patients with auricular fibrillation the apex rate and radial rate have been counted simultaneously in order to obtain the pulse deficit. In the few instances where the temperature of the patients has been increased, it is noted in the description of the patient.

#### CASES.

*Case 1 (Table I, Text-Fig. 1).*—P., male, photographer; age 46 years. Admitted March 26, 1917. Discharged April 30.

*Diagnosis.*—Myocarditis; arteriosclerosis; chronic nephritis; hypertension.

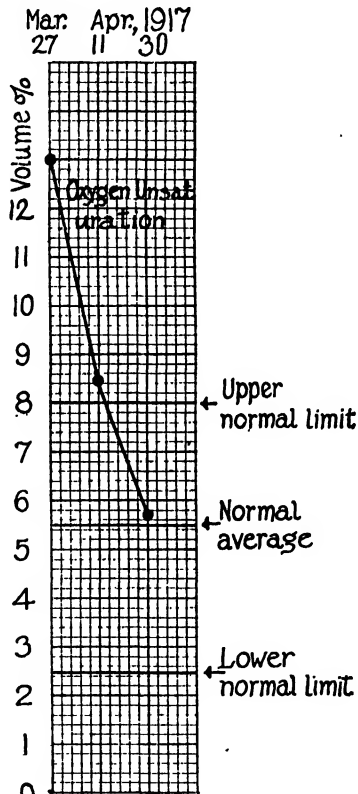
*Previous History, Symptoms, etc.*—Shortness of breath for a year, particularly on exertion; swelling of legs and abdomen for 2 months. No syphilis or rheumatic fever. He has used alcohol every day for several years.

*Physical Examination.*—Heart dullness and shadow on x-ray picture increased to the left and downwards. Limit of dullness is 3.5 cm. to the right and 14 cm. to the left of the middle line at the fourth interspace, 15 cm. to the left at the sixth interspace. An indistinct systolic murmur is heard all over the precordium. Aortic second sound is accentuated. Blood pressure: systolic 175, diastolic 125. There are moist râles and slight dullness at the lower parts of both lungs. He is cyanotic and the veins of the neck are distended. There is distinct ascites; the liver is felt, not tender. Marked edema of both legs extending up over sacrum. In the urine was found slight albumin, and occasionally granular casts. Blood urea considerably increased (73 mg. per 100 cc.). Index of urea excretion (McLean (4)) 15.0, in the course of the treatment increasing to 74.0. Wassermann test negative.

*Treatment.*—He was confined to bed and treated first with milk diet, and later with salt-free diet.

Three determinations of the oxygen unsaturation were done. The first was done the day after admission. He was distinctly incompen-sated and the value for the unsaturation was far above the upper normal

limit. The second determination was done at a time when it was doubtful whether he was compensated or not (Table I and Text-fig. 1). The oxygen unsaturation was at that time just above the upper normal limit. At the time of the third determination he was absolutely compensated



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of myocarditis; arteriosclerosis; chronic nephritis; hypertension.

at rest.<sup>2</sup> His oxygen unsaturation was at that time normal. The interpretation of this case is made difficult by the fact that there were quite a number of râles in the lungs at the time of the first determination, which may or may not have affected the saturation of the

<sup>2</sup> In the evening a slight edema around the ankles was found.

TABLE I.  
Case 1. *Myocarditis; Arteriosclerosis; Chronic Nephritis; Hypertension.*

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).		Oxygen unsaturation (a-v).	Clinical notes.									
	Date.	Condition.*	Sample 1.	Sample 2.†	Average (v).		vol. per cent	per cent		vol. per cent	Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.
1	1917		vol. per cent	vol. per cent	vol. per cent	per cent	per cent	per cent	vol. per cent	88	26	++	++	++	++	++	950	310	59.0
2	Mar. 27	In bed.	7.29	7.74	7.52	111	20.52	13	8.47	89	17	++	++	++	++	++	1,500	575	47.6
3	Apr. 11	½ hr.	14.69	14.67	14.68	125	23.15	8.47	89	17	++	++	++	++	++	++	1,500	625	51.8
	"	"	12.48	13.00	12.74	100	18.50	5.76	98	22	++	++	++	++	++	++	1,500	625	51.8

TABLE II.  
Case 2. *Mitral Stenosis and Insufficiency; Mitral Stenosis (?); Recurrent Endocarditis (?).*

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method). per cent	Calculated oxygen capacity (a).		Oxygen unsaturation (a-v). vol. per cent	Clinical notes.										
	Date.	Condition.	Sample 1. vol. per cent	Sample 2. vol. per cent	Average (v). vol. per cent		vol. per cent	per cent		Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.	
1	1917																			
2	Aug. 30	In bed.	4.50	5.00	4.75	71	13.13	8.38	108	34**	++	++	++	++	++	1,000	484	38.4		
3	Sept. 4	"	4.60	4.80	4.70	70	12.94	8.24	104	32	++	++	++	++	++	1,000	420	38.7		
4	" 7	"	2.30	2.20	2.25	67	12.38	10.13	100	28	++	++	++	++	++	1,000	740	39.7		
5	" 10	"	4.94	5.12	5.03	71	13.13	8.10	94	32	++	++	++	++	++	1,000	676	40.2		
6	" 12	"	3.62	3.80	3.71	71	13.13	9.42	90	24	++	++	++	++	++	1,000	565	39.8		
7	" 14	"	4.81	4.81	4.81	71	13.13	8.32	104	32	++	++	++	++	++	1,000	670	40.4		

\* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

† The interval between the two samples given in all the tables was generally about 15 to 20 minutes.

‡ The severity of these symptoms is indicated by means of crosses. Three stages are distinguished. Three crosses indicate a dyspnea (orthopnea) during which the patient was sitting almost straight up in bed in order to breathe. One cross means a just appreciable dyspnea, and two crosses mean an intermediate stage.

One cross for cyanosis means that only the lips were bluish, two crosses indicate that the ears and skin of the face were cyanotic, and three crosses mean a rather dark blue color of lips, face, and skin of the hands.

A just appreciable swelling of the veins in the neck and the dorsum of the hands is indicated by one cross. Distinct and prominent swelling of the veins of the neck, arms, and hands is indicated by three crosses. Two crosses represent an arbitrary medium stage.

As far as the liver is concerned, one cross means that it can be distinctly felt below the curvature; two crosses indicate that it extends 3 to 6 cm. downwards; and three crosses indicate that it extends still further, and is usually rather tender.

One cross for the edema indicates a little swelling on the dorsum of the foot around the ankles, or over the inside of the tibia. Two crosses indicate a distinct swelling of feet and legs, and three crosses indicate that the edema has extended up over the femurs and hips.

It will be understood that it has been difficult to obtain a satisfactory classification of these symptoms. A question mark indicates that in some instances it has been impossible to decide whether a symptom has been absent or present.

§ Some moist râles and slight dullness at the base of the lungs.

|| The lungs were clear except for a few crackling râles at the left axilla.

¶ Lungs clear.

\*\* Lungs clear at each determination.

arterial blood.<sup>3</sup> In several instances (Papers II and III) the oxygen unsaturation has been normal in patients, where just as many râles were heard as in this case. At the time of the second determination a few crackling râles in the left axilla were noticed. These disappeared the next day.

The edema in this case was supposed to be chiefly of nephritic origin. Besides this it was evident that he had an uncompensated myocarditis. The results of the oxygen determinations in this case at the different stages of the disease agree therefore with what was previously found in patients with compensated and uncompensated heart lesions (Papers II and III).

*Case 2 (Table II, Text-Fig. 2).—*M., male, student; age 14 years. Admitted August 28, 1917. Discharged October 21.

*Diagnosis.*—Mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis (?).

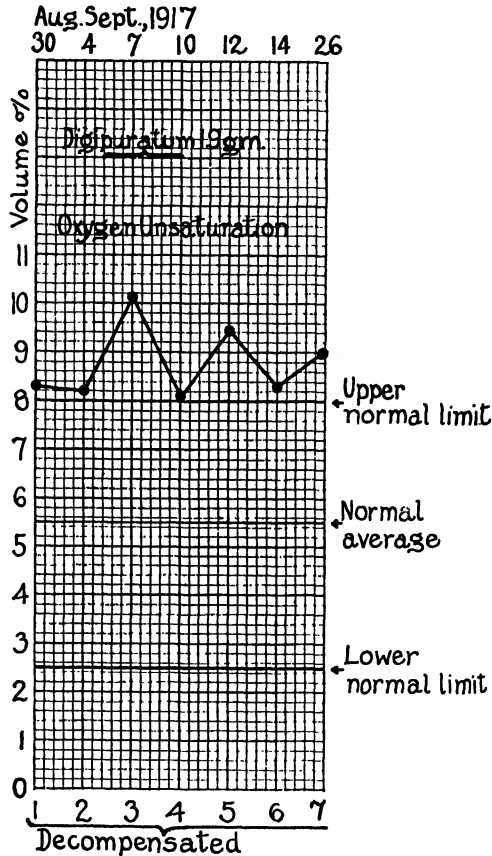
*Previous History, Symptoms, etc.*—Palpitation and dyspnea, particularly on exertion, for 3 years. He has had four attacks of rheumatic fever, the first 6 years ago. The second, 3 years ago, involved his heart. He had, furthermore, an attack of chorea 2 years ago. During the last 2 months, since the last hospital treatment for rheumatic fever and endocarditis, he has suffered from increasing palpitation and dyspnea. He has been confined to bed during the last 4 months.

*Physical Examination.*—A few râles on deep inspiration at the base of both lungs. The râles disappeared quickly and the lungs were clear at the time of the determinations of the venous oxygen. There is distinct bulging of the precordium. No thrills. The heart is considerably enlarged to the right and left, 4 cm. to the right and 12.5 cm. to the left of the middle line of sternum. The thorax is small. The x-ray picture shows a considerable enlargement in both directions. The normal heart sounds are replaced by blowing systolic and diastolic murmurs over the precordium. In the pulmonic area the pulmonic second sound can be heard, besides systolic and diastolic murmurs. The heart action is regular and rapid. The pulse is regular and small, collapsing in type. Blood pressure: systolic 116, diastolic 65. The electrocardiogram shows regular heart rhythm and signs of hypertrophy of the left ventricle. He is moderately cyanotic, the superficial veins are distended, and he is dyspneic. Liver extends from the fifth rib to 10 cm. below the costal margin in the nipple line, and is tender. Spleen is not felt. No ascites. There is slight edema of the lower legs and more distinct edema posteriorly over the hips. The edema over the hips disappeared quickly. Diuresis small, 420 to 740 cc. In the urine is found a trace of albumin; no casts.

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<sup>3</sup> See the discussion in Papers II and III.

*Treatment.*—He was confined to bed; had salt-free diet and restricted calories. From September 5 to September 10 he had digipuratum about 0.5 gm. a day, without any appreciable effect on the circulation. Besides, he had iron, arsenic,



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis.

and aspirin. His clinical condition as a whole was unchanged during the determination of the blood.<sup>4</sup> He ran some temperature at the time of determinations of the oxygen (rectal temperature between 100 and 101°F.).

<sup>4</sup> September 27. A determination of the blood oxygen (not given in the table) showed an unsaturation of 9 volume per cent. His condition was the same as before. October 21. He was discharged; his clinical condition was unchanged.



This is a case of uncompensated valvular heart disease on rheumatic basis. He probably has some endocarditis too. The values for the oxygen unsaturation are all above the upper normal limit. They are rather constant, all being between 8 and 10 volume per cent. The clinical condition was as a whole constant and unchanged, although the edema showed a tendency to disappear. It is important to emphasize that he was running a little temperature (100–101° F.) all the time. It is possible that this may have some effect on the oxygen unsaturation on account of increased metabolism.<sup>5</sup> His lungs were perfectly clear. It is interesting to note the constancy of the values for the oxygen unsaturation compared with those found in the three following cases (fibrillators). It is more constant than the values found in the normal individual (Papers I and II). The margin of action of his heart is probably very limited.<sup>6</sup> Digitalis was without effect—a striking contrast to what was found in the three fibrillators.

*Case 3 (Table III, Text-Fig. 3).—Z., housewife; age 54 years. Admitted June 21, 1917. Discharged July 25.*

*Diagnosis.*—Chronic cardiac disease (auricular fibrillation); chronic myocarditis.

*Previous History, Symptoms, etc.*—Shortness of breath and swelling of legs for the last 3 months. The symptoms have continuously grown more and more severe. Neither rheumatic fever nor lues.

*Physical Examination.*—A few râles during the last part of the inspiration on both sides from the eighth to the tenth rib. No distinct dullness. There is marked dyspnea, slight cyanosis, and distinct swelling of the superficial veins. Heart dullness from 3 cm. to the right of the middle line of the sternum to 14 cm. to the left in the fourth intercostal space. X-ray picture shows the heart moderately enlarged to the left. The heart action is very rapid and irregular. The electrocardiogram shows auricular fibrillation. No distinct murmurs are heard. The aortic and pulmonic second sounds are distinct and equal. The patient is generally a fibrillator. Blood pressure: systolic 185, diastolic 120. It is difficult

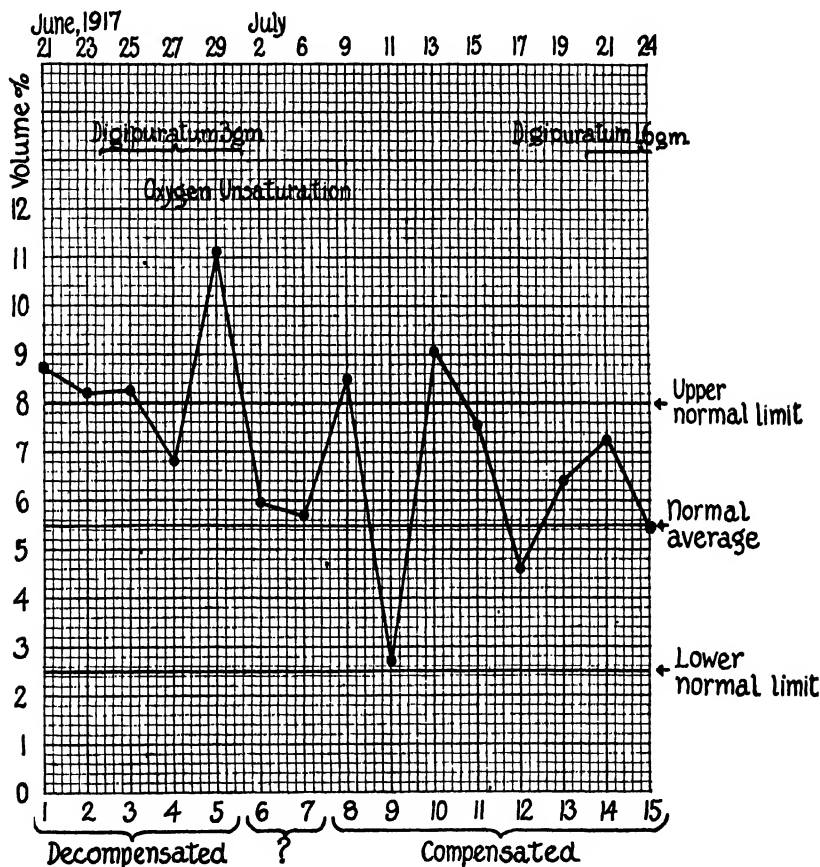
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<sup>5</sup> See the discussion in Paper II.

<sup>6</sup> As mentioned in Paper III, a valvular defect (aortic and mitral insufficiency, aortic stenosis) or increased blood pressure probably influences the action of the heart in the same way that a heavy wheel influences a machine. In mitral stenosis with auricular fibrillation the opposite holds true. The reason is that the increased resistance is here offered to the auricle instead of the ventricle (see the discussion on page 559, Lundsgaard (5), where the difference between the auricular and the ventricular form of heart failure is pointed out according to the result of the blood flow determinations reported in the paper referred to).

to decide whether or not the liver is enlarged. No tenderness over the left epigastric region. Spleen is not felt. No ascites. Wassermann test negative. Urine negative for albumin. Blood urea normal. Temperature normal.

*Treatment.*—She was confined to bed until July 14. From that date she was



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of chronic cardiac disease (auricular fibrillation); chronic myocarditis.

allowed to be out of bed, increasing the time every day. She had milk diet (800 cc.) for the 1st week. Since then salt-free diet (1,800 to 2,000 calories). Digipuratum, 0.5 gm. a day, was given from June 24 to 30 and about 0.3 gm. a day from July 20 to 25. She lost rapidly in weight from the 1st day in the hospital in spite of a rather small diuresis. Temperature normal.

TABLE III.  
Case 3. Chronic Cardiac Disease (Auricular Fibrillation); Chronic Myocarditis.

Determination No.	Bleeding.		Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Clinical notes.								
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).	Respirations.	Dyspnea.				Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	kg.		
1	1917 June 21	In bed.	13.83	13.84	13.83	122	22.57	8.74	91	44†	++	+	+	+	?	++			66.1
2	" 23	"	12.54	12.12	12.33	111	20.54	8.21	87	36†	++	+	+	+	?	++	1,200	635	64.6
3	" 25	"	11.85	11.43	11.64	107.5	19.89	8.25	110	36†	++	+	+	+	?	++	1,300	820	62.6
4	" 27	"	13.64	13.84	13.74	111	20.54	6.80	102	33	++	?	+	+	—	++	1,355	1,955	58.6
5	" 29	"	9.70	9.86	9.78	113	20.91	11.13	87	38	++	+	+	+	—	+	1,500	990	56.3
6	July 2	"	15.85	15.85	15.85	118	21.82	5.97	83	24	?	—	—	?	—	?	1,500	620	54.6
7	" 6	"	16.76	16.06	16.41	120	22.20	5.79	83	28	—	—	—	—	—	?	1,300	815	54.1
8	" 9	"	15.08	15.08	15.08	122	22.57	7.49	82	28	—	—	—	—	—	—	1,300	770	54.0
9	" 11	"	19.48	18.80	19.14	118	21.82	2.68	90	28	—	—	—	—	—	—	1,150	965	54.0
10	" 13	"	11.23	11.79	11.51	111	20.54	9.03	72	32	—	—	—	—	—	—	1,400	825	53.6

11	July 15	3 hrs.	12.10	12.10	106	19.62	7.52	$\frac{100}{92}$	28	-	-	-	-	1,300	980	52.6
12	"	17 1 hr.	14.66	14.66	104	19.25	4.59	?						1,300	800	53.0
13	"	19 1 "	12.82	12.82	(104)	(19.25)	6.43	$\frac{92}{68}$	30	-	-	-	-	1,300	400	53.2
14	"	21 1 "	11.26	11.02	100	18.50	7.36	$\frac{94}{82}$	30	-	-	-	-	1,300	450	52.8
15	"	24 2 hrs.	12.16	12.16	95	17.58	5.42	$\frac{87}{84}$	28	-	-	-	-	1,600	400	52.5

\* The upper figure is the apex pulse, the lower the radial pulse.

† A few râles at the base of the lungs.

‡ The lungs were clear from June 25 on.

This patient was distinctly uncompensated at admission, but recovered quickly (Table III). The values for the oxygen unsaturation of the venous blood started above the upper normal limit. At the time of the first two determinations the patient had râles in the lungs. At the third determination the lungs were clear and remained so during the rest of the time. In spite of the difference in the condition of the lungs no variations were encountered in the oxygen unsaturation of the venous blood of the first three determinations, a fact which supports what has previously been suggested (Paper II) that râles do not necessarily influence the oxygen unsaturation of the venous blood. This cannot mean anything else than that the saturation of the arterial blood has been as complete as in individuals with normal lungs. The fourth oxygen determination shows a value within normal limits; the clinical signs of incompen-sation were decreasing but still to be seen. The fact that a value for the oxygen unsaturation within normal limits can be obtained in a stage of clinical incompen-sation does not disagree with what has been previously found in Paper II.<sup>7</sup> This question will be discussed in the next case.

The fifth determination shows the highest values for the oxygen unsaturation obtained in this patient. The next day it went down to the normal average. At that time it was questionable whether she was clinically uncompensated or not. On July 7 it was evident that the clinical symptoms of incompen-sation had disappeared and from that date the oxygen unsaturation as a rule was within the normal limits. In two instances, however, it was above the upper normal limit and as a whole it was variable. The fact that we can obtain values for the oxygen unsaturation above the upper normal limit in a period of full compensation agrees with the findings in Patient 5, in Paper III, and with those in the two following patients in this paper. It is probably explained by the fact that the values for the oxygen unsaturation of the venous blood are above the upper normal limit in the uncompensated period and within normal limits in the compensated period. As in other fibrillators, however, we may

<sup>7</sup> In this publication all the determinations with compensated cases showed values within the normal limits, whereas all the values obtained in the uncompensated cases were above. See the discussion in Paper II.

find abnormally high values in the period of compensation. The relation of the curve for the oxygen unsaturation to the digitalis treatment is unmistakable, and the same is found in the following patients and discussed more in detail.

*Case 4 (Table IV, Text-Fig. 4).—D., housewife; age 37 years. Admitted July 19, 1917. Discharged October 14.*

*Diagnosis.*—Mitral insufficiency and stenosis; auricular fibrillation.

*Previous History, Symptoms, etc.*—Palpitation and shortness of breath, particularly on exertion. Swelling of legs. She had rheumatic fever 14 years ago, but had no heart trouble at that time. Never had lues. For the last 2 years she has suffered from her present symptoms, periodically, not continuously. 3 weeks ago she left a hospital in fairly good condition, but her symptoms reappeared quickly and have steadily grown worse. ●

*Physical Examination.*—No cyanosis or dyspnea. There is swelling of the superficial veins. The heart dullness is considerably increased, extending from the fifth interspace, 6 cm. to the right and 14 cm. to the left of the middle line of the sternum. The x-ray picture shows the heart shadow correspondingly increased. No thrill. A harsh systolic murmur, replacing the first sound, is heard over the precordium and transmitted upward in the direction of the left axilla. An indistinct presystolic murmur is heard at the apex. The pulmonic second sound is accentuated and reduplicated. All the sounds at the bases are indistinct. The heart action is violent and irregular. The electrocardiogram shows auricular fibrillation. Systolic blood pressure 100 (palpation). Diastolic could not be determined. Liver is felt 10 cm. below the curvature in the nipple line; tender. Spleen not felt. No ascites. Marked edema extending upward over the hips. Urine negative for albumin. Wassermann test negative.

*Treatment.*—She was confined to bed and treated with milk diet (800 cc.) during the first 10 days. Later she had salt-free diet (1,800 to 2,200 calories). Besides iron, she had 1.6 gm. of digipuratum from August 22 to 26 (about 0.3 gm. a day). Later she had 1.6 gm. of digipuratum from August 27 to September 10 (about 0.1 gm. a day.) Her temperature was normal during all the determinations.

At the time of the first three determinations some râles were heard at the base of both lungs. Later on the lungs were clear. Thirty determinations of the oxygen unsaturation of the venous blood were done during two periods with an interval of 2 weeks between. In spite of the uncompensated condition and the râles the values for the oxygen unsaturation were within normal limits the first five times. The first four determinations gave values which almost followed the normal average, the fifth was nearer the upper limit. The results

TABLE IV.  
Case 4. *Mitral Stenosis and Insufficiency; Auricular Fibrillation.*

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Folmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Clinical notes.								
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.
1	1917 July 19 ½ hr.		vol. per cent 8.30	vol. per cent 8.56	vol. per cent 8.43	per cent 78	vol. per cent 14.43	vol. per cent 6.00	72	—	—	+	++	++	1,750	333	68.4
2	" 20 In bed.		8.73	8.95	8.84	79	14.61	5.77	78	—	—	+	++	++	1,750	333	
3	" 21 "	"	9.51	9.51	9.51	82.5	15.28	5.77	69	—	—	+	++	++	1,025	280	
4	" 24 "	"	8.33	8.73	8.53	78	14.43	5.90	80	—	—	+	++	++	1,200	240	67.6
5	" 26 "	"	8.25		8.25	82.5	15.28	7.03	70	—	—	+	++	+	1,200	820	67.8
6	" 28 "	"	6.72	6.30	6.51	82.5	15.28	8.77	56	—	—	+	++	+	1,200	3,150	63.7
7	" 29 "	"	11.16	10.72	10.94	84	15.53	4.59	52	—	—	?	++	+	1,200	2,825	51.2
8	" 30 "	"	10.53	10.53	10.53	82.5	15.28	4.75	44	—	—	?	+	?	1,200	1,275	58.8
9	" 31 "	"	11.17	11.17	11.17	82.5	15.28	4.11	58	—	—	—	?	?	1,200	630	57.2
10	Aug. 1	"	9.11	8.73	8.92	82.5	15.28	6.36	48	—	—	—	—	—	1,200	555	55.0
11	" 2 "	"	9.17	9.15	9.16	87	16.10	6.94	52	—	—	—	—	—	1,200	525	54.1

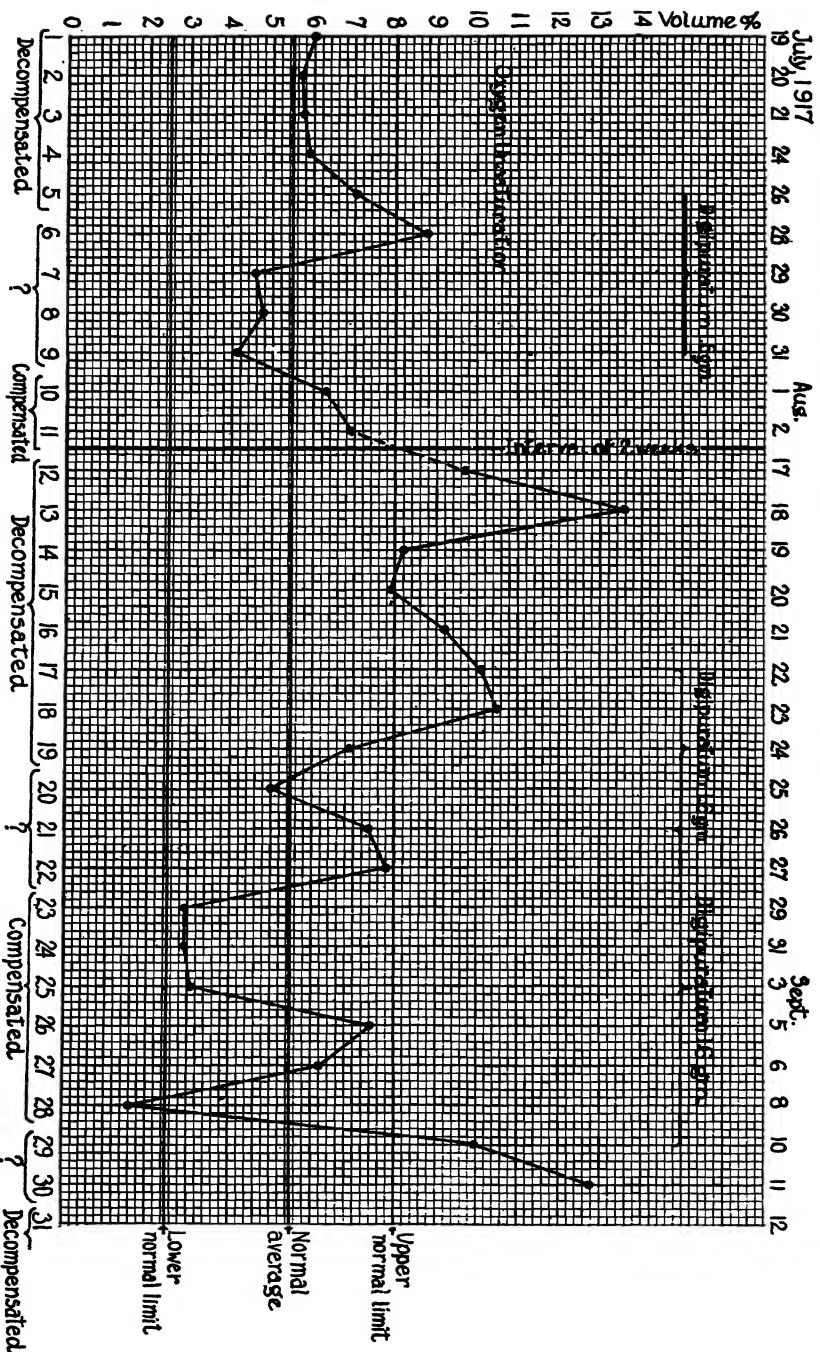
Interval of 14 days.

12	Aug. 17	In bed.	5.89	5.89	5.89	84	15.53	9.64	99	22	+	?	?	—	1,200	760	57.7
13	"	"	1.97	2.02	2.00	84	15.53	13.53	88	24	++	+	+	?	1,200	730	57.7
14	"	"	7.35	7.35	7.35	84	15.53	8.18	78	20	+	?	?	?	1,200	700	57.6
15	"	"	7.40	7.84	7.62	84	15.53	7.91	76	20	+	?	?	?	1,200	615	58.2
16	"	"	6.84	7.04	6.94	87	16.10	9.16	60	24	+	?	+	+	1,200	834	58.4
17	"	"	6.05	6.05	6.05	87	16.10	10.05	92	24	+	+	+	+	1,200	555	58.7
18	"	"	4.88	5.22	5.05	84	15.53	10.48	84	30	++	+	+	+	1,250	645	58.6
19	"	"	8.55	8.13	8.34	82.5	15.28	6.94	85	24	+	?	?	+	1,200	993	59.2
20	"	"	9.35	9.55	9.45	78	14.43	4.98	88	24	?	?	?	?	1,200	940	59.0
21	"	"	7.16	7.16	7.16	78	14.43	7.27	72		—	—	—	?	1,200	1,280	59.1
22	"	"	6.34	6.54	6.44	77	14.22	7.78	68	22	—	—	—	—	1,200	1,130	59.0
23	"	"	11.86	11.86	11.86	80	14.79	2.93	60	20	—	—	—	—	1,200	1,000	58.6
24	"	"	12.35		12.35	82.5	15.28	2.93	64	22	—	—	—	—	1,200	1,250	58.5
25	Sept. 3	"	12.46	12.54	12.50	84	15.53	3.03	63	20	—	—	—	—	1,200	945	58.4

\* Few rales.

† The lungs were clear from July 24 on.





Text-Fig. 4. Oxygen unsaturation of venous arm blood. Case of mitral stenosis and insufficiency; auricular fibrillation.



of the first five oxygen determinations show the same things which were seen once in Case 3: a normal oxygen unsaturation at a time when the patient is distinctly clinically incompensated. This is probably an expression of the fact that she was improving. It will be seen from the chart that the severity of the symptoms of incompensation were decreasing. The fourth and fifth determinations showed an increasing tendency. Digitalis treatment was started on July 26. The oxygen unsaturation was still higher the next day (No. 6), this time above the normal limit. 1 day later a sudden drop in the oxygen unsaturation (4.6 volume per cent) occurred. Whether or not this was a result of the digitalis treatment cannot be definitely decided from the present facts. Nos. 8, 9, 10, and 11 were all within the normal limits. The oxygen determinations then had to be stopped for 2 weeks. At the time of the tenth and eleventh determinations she was clinically compensated; she felt well and was allowed to be out of bed.

This clinically compensated condition lasted for about 12 days. At that time (August 14) a rather sudden change in condition occurred. The patient began to feel uneasy. She had palpitations, throbbing in the veins of the neck, and pain in the left hypochondrium. On August 17 when the oxygen determinations were taken up again she was slightly incompensated. The oxygen unsaturation (9.7 volume per cent) was above the upper normal limit. The next day it was still higher (13.5 per cent), and the clinical symptoms of incompensation were more marked. The two following determinations (Nos. 14 and 15) showed a considerable drop in the oxygen unsaturation which went down to the upper normal limit. However, an increase appeared again and the incompensation seemed to be growing. Digitalis treatment was therefore instituted (August 22). 2 days later a sudden drop occurred in the oxygen unsaturation, almost identical with what was seen at the time of the first digitalis treatment. The clinical condition now improved rapidly. On August 25 it was impossible to decide whether she was incompensated or not, and 4 days later she was again in full clinical compensation. On August 24 and 25 the same condition was encountered as at her admission: normal oxygen unsaturation despite incompensation. However, as can be seen from the table, the clinical incompensation is rapidly dis-

appearing. On August 26 the digitalis dose was decreased to 0.1 gm. a day, instead of 0.5 gm. For 10 days she felt well and was compensated in rest. On September 10 she began to feel uneasy again. Her pulse rate and pulse deficit increased, and signs of incompensation appeared. The digitalis was stopped September 11 for a week. Her clinical condition grew rapidly worse and on September 12 she was again incompensated. Corresponding to the change in the clinical symptoms, a sudden large increase appeared in the oxygen unsaturation, which by September 10 was 10 volume per cent and on September 11, 12.8, which is far above the upper normal limit. The oxygen determinations had to be stopped at that time. A week later she was given digipuratum in larger doses, 0.5 gm. a day, and the symptoms of incompensation disappeared again. The oxygen determinations in this patient show: (1) that normal values for the oxygen unsaturation of the venous blood may be found with râles in the lungs; (2) that normal oxygen unsaturation may be associated with full incompensation but improving clinical condition; (3) that an increase in the oxygen unsaturation to above the upper normal limit may precede the clinical symptoms of incompensation; (4) the results suggest the oxygen unsaturation as an indicator of the action of digitalis;<sup>8</sup> (5) great variations in the oxygen unsaturation are met with in this patient, as in previous fibrillators.

*Case 5 (Table V, Text-Fig. 5).—D., male, paper maker; age 31 years. Admitted February 9, 1917. Discharged May 5.*

*Readmitted June 19. Discharged July 25.*

*Readmitted August 22. Discharged October 2.*

*Diagnosis.*—Mitral stenosis and insufficiency; auricular fibrillation; diabetes mellitus; slight chronic interstitial nephritis.

*Previous History, Symptoms, etc.*—Shortness of breath for 3 years before his admission to this hospital. At 9 years of age he had rheumatic fever without heart symptoms. Denies lues. After the heart symptoms had first started 4 years ago they grew continuously worse. Besides shortness of breath and palpitation he often had swelling of the lower extremities. He has been admitted to the hospital several times (four) in an incompensated condition and every time it has been more difficult, and taken longer to obtain compensation. Be-

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<sup>8</sup> The relation of the pulse rate and pulse deficit to the oxygen unsaturation suggests indirectly the same thing.

sides his heart disease he had a mild, not progressive diabetes and slight chronic interstitial nephritis.

*Physical Examination.*—The local physical signs of heart disease remained constant. At the first admission he had a considerable enlargement of the heart dullness 15.5 cm. to the right of the middle line of the sternum and 17.5 cm. to the left in the fifth intercostal space. No thrill. The x-ray picture showed corresponding enlargement of the heart shadow. At the apex a systolic and a diastolic murmur were heard. A systolic murmur was heard at the bases and the pulmonary second sound was accentuated. The pulse was irregular. The electrocardiogram showed arrhythmia perpetua. Blood pressure: systolic 180; diastolic 140. Wassermann test negative. In urine a trace of albumin, hyaline, and granular casts. Blood urea normal. Index of urea excretion fairly normal; now and then somewhat diminished. On ordinary mixed diet he would show sugar in the urine and about 0.20 per cent sugar in blood.

*Treatment.*—He was treated with digipuratum, antidiabetic and salt-free diet. Until he was absolutely compensated he was confined to bed. Now and then he had a slight increase of temperature (100–100.5°F.).

Forty-eight determinations of the oxygen unsaturation were done at three different periods, at the three successive admissions.

*Period I.*—Two determinations were done (February 17 and March 8). He had at that time been treated and the symptoms of incomensation had disappeared and he was fully compensated, at rest. The values were normal (4 and 5 volume per cent).

*Period II.*—On June 19 he was readmitted to the hospital in a severely uncompensated condition (dyspnea, cyanosis, distended veins, swelling of liver, and edema, as shown in Table V). Some râles were heard at the base of the lungs at that time. The temperature was slightly increased (100–102°F.) on the first 2 days; later normal. The determinations of the oxygen unsaturation showed considerably increased values. The first eight (the 1st week in the hospital) are all above the upper normal limit. He was at that time uncompensated but improving. At the time of the first seven determinations (Nos. 3 to 9) moist râles were heard at the base of both the lungs. At the time of the eighth determination (No. 10) his lungs were clear. No difference is seen in the value for the oxygen unsaturation. The next four determinations (Nos. 11 to 14) show varying values—two above and two below the upper normal limit. A question mark on the diagram indicates that it was difficult to decide whether the patient was clinically compensated or



10 June 27	In bed.	8.21	8.21	8.21	101	18.70	10.49	71 70	28*	++	+	+	?	?	1,100	1,385	60.4
11 " 28	"	13.44	13.24	13.34	108	19.96	6.62	68 65	22*	+			?	?	1,100	1,840	60.8
12 " 29	"	6.20	6.18	6.19	106	19.62	13.43	?	—*	+	?		—	?	1,100	902	58.9
13 " 30	"	14.65	14.65	14.65	111	20.52	5.87	?	23*		—		—	—	1,100	1,090	58.9
14 July 1	"	11.34	10.92	11.13	112	20.72	9.59	75	24*		—		—	—	1,100	859	58.4
15 " 2	"	12.72	12.42	12.57	116	21.45	8.88	66 69	25*		—		—	—	1,100	785	58.0
16 " 3	"	15.56	15.56	15.56	116	21.45	5.89	68 74	24*		—		—	—	1,400	960	57.6
17 " 5	"	10.54	11.48	11.01	116	21.45	10.44	69	28*		—		—	—	1,400	1,080	57.6
18 " 7	"	15.42	16.60	16.01	116	21.45	5.44	61 59	28*		—		—	—	1,400	1,880	57.3
19 " 9	"	9.00	9.00	9.00	116	21.45	12.45	57 78	28*	As a rule dyspnea only on exertion.			—	—	1,400	1,950	57.4
20 " 10	3 hrs.	9.38		9.38	117	21.64	12.26	66 72	28*				—	—	1,400	1,820	57.2
21 " 12	O.N.†	17.50	17.50	17.50	117	21.64	4.14	67	26*				—	—	1,400	1,620	57.2
22 " 13	"	15.55	15.77	15.66	111	20.52	4.86	66 64	26*		—		—	—	1,400	1,140	57.2
23 " 14	"	9.91	9.01	9.46	104	19.25	9.79	64 68	26*		—		—	—	1,400	840	57.2
24 " 16	‡ hr.	14.08	14.52	14.30	106	19.62	5.32	78 77	30*		—		—	—	1,400	1,035	57.4

\* Lungs clear.

† A few râles at the base of the lungs.

‡ O. N. (over night in bed) means that the blood was drawn in the morning; ‡ hr. means that the patient had been in bed

‡ hour before the blood was drawn.

TABLE V—*Concluded.*

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Faj. mer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Clinical notes.									
	Date	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
1917			vol. per cent	vol. per cent	vol. per cent	per cent	vol. per cent	vol. per cent										
25	July 18	1 hr.	16.56	16.52	16.54	106	19.62	3.08	80	30*					1,400	1,140	57.5	
26	" 20	1 "	13.32	13.32	13.32	106	19.62	6.30	72	—*	As a rule dyspnea only on exertion.				1,400	1,415	57.4	
27	" 23	O.N.	9.46	9.46	9.46	105	19.44	9.98	?	24*						1,400	760	57.4
28	" 24	1 hr.	13.67	13.42	13.55	105	19.44	5.87	86	32*						1,400	530	57.7
Third admission.																		
29	Aug. 22	3 hr.	11.08	11.08	11.08	111	20.52	9.44	88	38§	++	++	++	++				
30	" 22	In bed.	7.66	8.08	7.87	(111)	(20.52)	12.65	78	100	36§	++	++	++	++			
31	" 23	"	10.02	10.02	10.02	110	20.36	10.34	92	100	32§	++	++	++	++	1,500	515	
32	" 23	"	8.92	8.92	8.92	(110)	(20.36)	11.44	78	108	34§	++	++	++	++		70.8	
33	" 24	"	10.17	10.53	10.35	110	20.36	10.01	98	96	44§	++	++	++	++	1,500	680	
34	" 25	"	8.50	9.30	8.90	110	20.36	11.46	88	94	30§	++	++	++	++	1,500	665	
35	" 26	"	8.96		8.96	106	19.62	10.66	92	89	28§	++	++	++	++	1,500	2,475	
									74			++	++	++	++		71.4	
												++	++	++	++		70.6	
												++	++	++	++		69.0	



36 Aug. 27	In bed.	11.46	11.46	106	19.62	8.16	72	28§	++	+++	++	++	++	++	1,500	2,100	67.8
37 "	"	9.38		9.38	107.5	10.50	68	28§	++	+++	++	++	++	++	1,075	1,344	
38 "	"		12.07	12.07	107.5	7.81	80	32§	++	++	++	++	++	++	1,400	315	
39 "	"		12.30	11.80	107.5	7.78	62	32§	++	++	++	++	++	++	1,600	485	64.4
40 "	"		4.57	4.57	107.5	15.31	68	32§	++	++	++	++	++	++	1,600	750	64.7
41 Sept 1	"		12.65	12.65	107.5	7.23	63	28§	++	++	++	+	+	++	1,500	1,665	64.4
42 "	"		4.92	4.68	106	14.82	64	32§	++	++	+	+	+	++	1,500	522	62.3
43 "	"		11.52	11.72	106	8.0	74	30§	+	?	?	+	+	++	1,500	1,375	62.7
44 "	"		7.67	7.67	106	11.95	66	32§	+	++	+	+	+	+	1,500	1,890	63.4
45 "	"		11.25	11.25	103	7.81	70	28* }		-	-	+	+	+	1,500	3,650	60.4
46 "	"		14.00	14.00	104	5.25	78	24*	Only on exertion.	-	-	+	+	?	1,500	2,125	58.3
47 "	"		13.00	13.00	104	6.25	64	24*		-	-	?	?	-	1,500	2,840	55.7
48 "	"		11.86	11.92	104	7.36	70	24*		-	-	-	-	-	1,500	2,935	54.7

§ A few rather coarse rales at the left base; no distinct dullness.

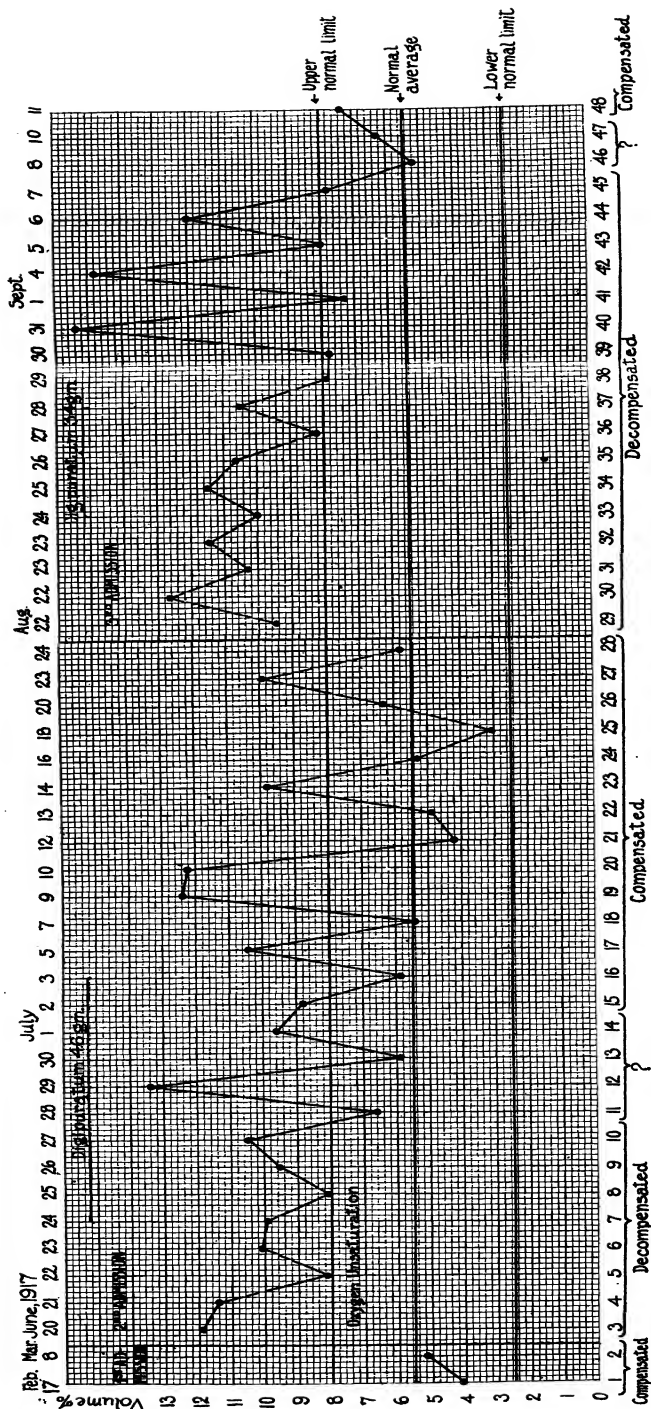
not. On July 2 (determination 15) a period of clinical compensation at rest began. The values for the oxygen unsaturation show wide variations. Most of them are within the normal limits; a considerable number, however, are above the upper normal extreme. Upon the whole the values for the oxygen unsaturation are becoming lower and lower and there is a greater tendency to variations during the compensated than during the severely uncompensated period. A line on the diagram shows the digitalis treatment (about 0.5 gm. of digipuratum a day from June 24 to July 3). It can be seen from the table that the edema decreased rapidly before the severity of the other symptoms of uncompensation lessened. The same has been seen in other instances.

*Period III.*—A month later he was readmitted to the hospital. He had then for a week been in a condition of rapidly increasing uncompensation which on admission was more severe than before. He was much more dyspneic and very cyanotic. The veins of the neck and arms were greatly distended and the edge of the liver was felt 10 cm. below the costal curvature in the nipple line. Heavy edema was noticed extending up his legs over the hips. The diuresis was small and he felt much distress. He was confined to bed and treated first with milk (800 cc. a day) for a week and later with salt-free diet (1,500 to 2,000 calories). From August 24 to 28 he had in all 3.4 gm. of digipuratum. It was then stopped on account of extrasystoles.<sup>9</sup> From September 1 to 3 he had 3 gm. of diuretin. The uncompensated stage lasted this time longer than before (from August 22 to September 8). Seventeen determinations were done in this period. The first nine were all above the upper normal limit, occupying a rather limited space. The next ten were extremely variable, some of them going a little below the upper normal line; most of them, however, were far above.

His condition was only very slowly improving (Table V) until September 7 when a marked change took place. After 2 or 3 days

<sup>9</sup> The extrasystoles occurred regularly alternating with the ordinary ventricular beats. Only every other ventricular contraction was felt at the wrist. The apex rate was 160, the radial rate 80 (August 28, determination 37). The peculiar condition of the heart rhythm does not seem to have had any influence on the oxygen unsaturation of the venous blood (see Table V and Text-fig. 5).





TEXT-FIG. 5. Oxygen unsaturation of venous arm blood. Case of mitral stenosis and insufficiency; auricular fibrillation.



he became absolutely compensated at rest and felt extremely well. On the same date a considerable drop occurred in the oxygen unsaturation, which in the 5 following days (the last four determinations) was within normal limits. The oxygen determinations were stopped September 11. The patient was discharged October 2. He felt well and was clinically compensated. It will be seen from the table that râles were heard in the lungs from his admission (August 22) to September 6 (determination 44). From September 7 his lungs were clear. The first 4 days of his third admission he ran some temperature (100–101° F.). Thereafter his temperature was normal.

The values for the oxygen unsaturation in this patient agree as a whole with those previously found in patients with uncompensated heart disease and auricular fibrillation. We can distinguish three periods in the course of the disease: (1) A period of severe uncompensation. The values for the oxygen unsaturation are practically all above the upper normal limit, varying only to a moderate degree. (2) A transitory period where the symptoms of uncompensation are disappearing or doubtful. In this period the values for the oxygen unsaturation are extremely variable, some above and some below the upper normal limit. (3) A period of clinical compensation at rest in which the oxygen unsaturation as a whole has a tendency to fall within the normal limits. Now and then, however, as seen in Patient 5, Paper III, the unsaturation exceeds the upper normal limit, a condition which never has been observed in compensated non-fibrillators.

#### DISCUSSION.

*Relation of Oxygen Unsaturation to Clinical Symptoms.*—As a whole, it can be said that the determinations of the oxygen unsaturation in the venous arm blood are of value in the diagnosis and treatment of patients with symptoms of circulatory disturbances. There is undoubtedly a close relation between the extent of the oxygen unsaturation and the clinical condition of the patients. As a whole, we have seen that the oxygen unsaturation in patients with compensated heart disturbances has values within the limits found in normal individuals, whereas patients with uncompensated heart lesions show values above the upper normal extreme.

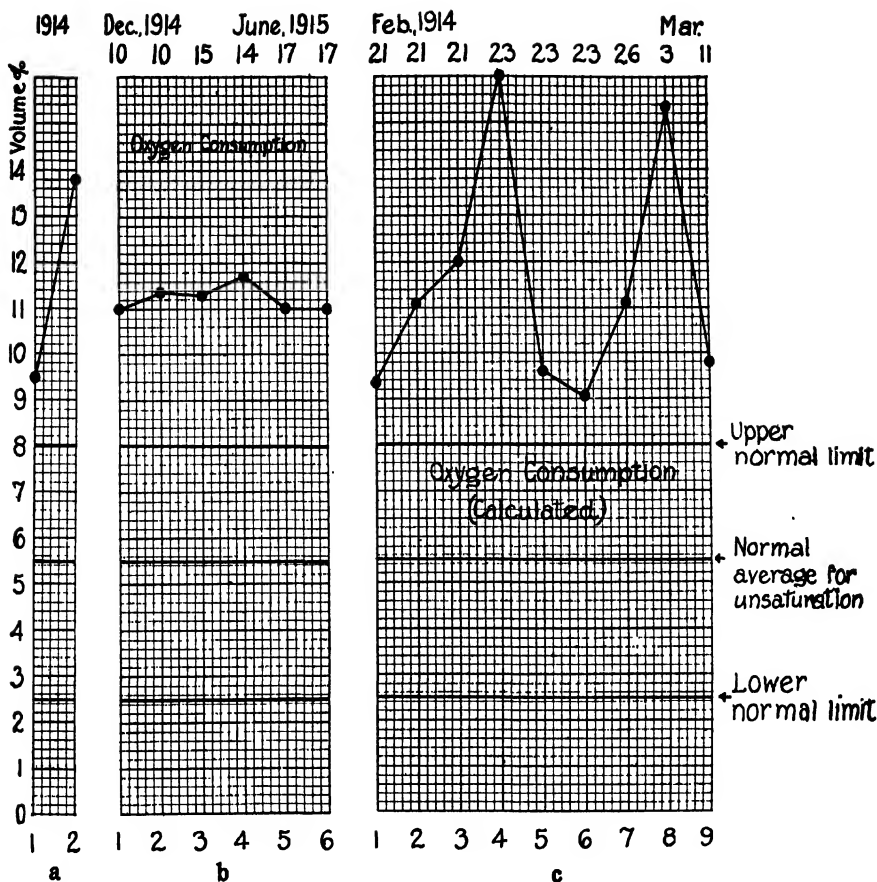
In patients in whom the uncompensated condition of the circulation is decidedly improving we may find normal values for the oxygen unsaturation preceding the compensated stage, and we have seen an increase in the oxygen unsaturation to above the upper normal limit occur where uncompensation was developing, but not yet clinically distinct.

In patients with auricular fibrillation the oxygen unsaturation may oscillate widely and show on some days values above the normal maximum, even when no uncompensation is developing.

It is seen from the tables that the different clinical symptoms of uncompensation differ in their relation to the oxygen unsaturation. Cyanosis and swelling of the superficial veins are closely related. Dyspnea appears to be usually accompanied in heart patients by increased oxygen unsaturation, but it is of course known also to occur in conditions, such as acidosis, in which the circulation is probably not retained. Edema seems to be to a great extent dependent upon special cases. It was usually possible to decrease the edema materially by the diet (salt-free) without interfering with the other symptoms and without causing any change in the oxygen unsaturation.

*Oxygen Unsaturation and Blood Flow.*—An attempt to calculate the rate of circulation (the minute volume of the heart) from the data obtained by the oxygen determination is not yet justified. Several facts, however, suggest that variations in the output from the heart have been by far the most important cause for the variations in the oxygen unsaturation in the patients. In Papers II and III a comparison was drawn between the directly determined oxygen unsaturation and the values for the oxygen consumption<sup>10</sup> calculated from blood flow determinations by means of Krogh and Lindhard's nitrous oxide method (5). A close parallelism was found, as far as the compensated patients were concerned,—even the peculiarities in the fibrillators were encountered by both methods. A similar close agreement is seen in patients with uncompensated circulatory disturbance. Text-fig. 6, *a*, *b*, and *c* represents the calculated oxygen consumption in three patients with uncompensation. It will be seen that all the values thus calculated on uncompensated patients

<sup>10</sup> The difference between oxygen unsaturation and oxygen consumption is defined in Paper II.



TEXT-FIG. 6, *a*, *b*, and *c* (*a*). Case of aortic insufficiency and stenosis; mitral insufficiency; uncompensated.<sup>11</sup> Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method. The normal average and the normal extremes for oxygen unsaturation are indicated.

(*b*) Case of aortic and mitral insufficiency and stenosis; auricular fibrillation.<sup>12</sup> Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

(*c*) Case of mitral stenosis and insufficiency; auricular fibrillation; uncompensated.<sup>13</sup> Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

<sup>11</sup> Patient 8, Lundsgaard (5), p. 537.

<sup>12</sup> Patient 2, Lundsgaard (5), p. 521.

<sup>13</sup> Patient 3, Lundsgaard (5), p. 524.



by the Krogh and Lindhard method are above the upper limit for the directly determined oxygen unsaturation in normal subjects. The patients in Text-fig. 6, *b* and *c* (Krogh and Lindhard's method) were fibrillators. The patient in Text-fig. 6, *c*, shows extremely variable values, but they are all above the upper normal limit. She was at the time of the determination in a continuously uncompensated stage.

It seems, therefore, justifiable to expect that further investigations of the oxygen unsaturation in patients with different forms of circulatory and pulmonic disturbances may give us sufficient data to allow a conclusion concerning the relation of the oxygen unsaturation to the circulation.

The results on fibrillators suggest strongly that there is a qualitative difference in the dynamic pathogenesis of the heart failure in patients with normal rhythm of the heart and in patients with abnormal rhythm. This has been previously pointed out and discussed by the writer.<sup>14</sup>

*Oxygen Unsaturation and Digitalis Action.*—It seems promising to apply the oxygen determination to the study of the action of drugs, particularly digitalis, in the circulation. Cohn and Fraser have shown (6) that digitalis in a certain quantity will alter the T wave in the ventricular complex of the electrocardiogram, and have given us a valuable means of detecting the appearance of the local action of digitalis. The relation of the oxygen unsaturation to the digitalis treatment in the case reported here seems to indicate that the determination of the blood oxygen may be used as an indicator of the appearance of the effect of digitalis on the circulation as a whole.

#### SUMMARY.

1. A report is made of 103 determinations of the oxygen unsaturation of the venous blood of five patients with uncompensated heart diseases.

2. Values for the oxygen unsaturation within normal limits were found only under two circumstances: (*a*) in a stage of full compensation, and (*b*) in a stage of incompensation where the symptoms were rapidly lessening.

<sup>14</sup> Lundsgaard (5), pp. 549–560.

3. Values above the upper normal extreme were met with under three circumstances: (a) during incompensation, (b) during compensation just before the clinical symptoms of incompensation had developed, and (c) at times in patients with auricular fibrillations in a condition of complete and stable compensation.

4. A comparison has been drawn between the directly found value for the oxygen unsaturation and the values for the oxygen consumption calculated from previous experiments by the writer on the blood flow (minute volume of the heart), in patients with similar clinical conditions. A close agreement existed.

5. It seems probable from our experience with patients under digitalis therapy that the oxygen unsaturation affords an objective criterion of the positive effect of the therapy.

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<sup>15</sup> An extensive bibliography is found in the first two papers of this series (1, 2).



## STUDIES OF ACIDOSIS. X.

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The present paper includes a reply to Barnett's (1) critique in the preceding article and a summary showing the nature of the results obtained with the methods for estimating alkaline reserve from urine and alveolar air analyses.

Fitz and the writer (2) succeeded recently in demonstrating a quantitative relationship between the alkaline reserve and the excretion of acids in excess of fixed base, as measured by ammonia plus titratable acid in the urine. The empirical formula utilized to demonstrate the relationship was,  $\text{plasma CO}_2 = 80 - \sqrt{\frac{D}{W}} \sqrt{C}$ ,  $D$  representing the cc. of 0.1 N titratable acid and ammonia excreted per 24 hour time unit, and  $C$  (concentration) the amount excreted per liter.

Barnett states the belief that this formula does not express the relationship between plasma bicarbonate and acid excretion with sufficient accuracy to justify its use. The reason for this opinion is that the results show that the average error in calculating the plasma bicarbonate from the excretion exceeds that which would result from maximal analytical errors in all of the determinations involved. This seems to us insufficient ground for the criticism. The essential question, for our purpose, is not whether the plasma bicarbonate can be estimated from urine excretion with no error except that of the chemical determinations, but whether the total sum of the errors of analysis, individual variation in kidney function, and fault in the empirical formula is within such limits that the excretion may be of any assistance in estimating the alkaline reserve when conditions prevent the direct determination of the latter in the blood.

We have given in Papers IV (2) and VI (3) data which show the limits of accuracy encountered in estimating plasma bicarbonate from acid excretion in practically every form of diabetic acidosis. It does not appear to us that a valid reason has been advanced for modifying the conclusion based on those data; *viz.*, that the ammonia plus acid excretion is quantitatively related to the bicarbonate deficit in the blood, and that the relationship is sufficiently uniform to be useful in estimating this deficit when the limitations of accuracy, as shown by our data, are taken into consideration.

Barnett's claim that all the variables of the formula except the two most important ones, the blood bicarbonate and ammonia excretion, may be replaced by a constant without significantly diminishing the average accuracy of the formula, would likewise appear to us, even if entirely justified, to detract nothing from the correctness of the above conclusion.

Any simplification in the calculation not resulting in loss of accuracy would be an improvement, however, and therefore it is desirable to examine somewhat closely the formula minus one, two, and three of its variables in order to decide how many of them may be deleted without lessening the reliability of results.

Concerning the dropping of  $C$ , thereby simplifying the formula

$$\text{Plasma CO}_2 = 80 - \sqrt{\frac{D}{W}} \sqrt{C} \text{ to, Plasma CO}_2 = 80 - 5 \sqrt{\frac{D}{W}},$$

we agree with Barnett that it makes the equation not only simpler, but also measurably more accurate and is therefore in every way desirable.<sup>1</sup> In preliminary experiments in which we tested several

<sup>1</sup> From the negligible average influence of  $C$  it appears that, at least as long as either normal or exceptionally high volumes of urine are excreted (which was the case with all our patients), variations in the volume are of no effect on the acid excretion. We are not, however, prepared to follow this conclusion as to the non-effect of  $C$  to its logical limit and state that in a patient with severe diabetic acidosis flushing with water is of no value. A liter of urine is not an abnormally small amount in a normal adult, but it could carry out only about 15 gm. of  $\beta$ -hydroxybutyric acid, if, as indicated by the data of Magnus-Levy and others, this acid is never excreted in concentration greater than 1.5 per cent. To remove the much larger amounts sometimes formed, excretion of several liters of urine per 24 hours appears necessary. The formation of large amounts of acetone bodies,

formulas before one was chosen to apply systematically, the value of  $C$  appeared to have an appreciable effect, although much less than that of  $D$ ; *i.e.*, other conditions being the same, a greater volume of urine appeared to carry out somewhat more acid than a smaller volume. We accordingly indicated this effect by introducing  $C$  into the formula, and indicated its comparatively minor importance by using its fourth root. The formula thus involved enabled us to solve our main problem; *viz.*, the question as to whether any quantitative relationship could be demonstrated between fall in alkaline reserve and rise in ammonia and acid excretion. The formulas which had been discarded in the preliminary tests we did not afterwards apply to the main body of our data. It is fortunate that Barnett has now retested the formula without  $C$  on our published data, and shown that this variable may be neglected. With the formula thus simplified it becomes possible, as indicated in the accompanying table, to interpret the 24 hour excretion of acid plus ammonia directly into terms of acidosis with the use of no more elaborate terms than  $\frac{D}{W}$ , or cc. of 0.1 N acid plus ammonia per kilo.

Barnett also calculates from the results of Paper IV that but little is gained in average accuracy by including variations in the body weight in the estimation. The data of Paper IV, however, taken without those of Paper VI, are not suited to decide statistically the question of the influence of body size. Of the 65 determinations on diabetics reported in Paper IV, 29 are on a single patient of 50 kilos weight, and of the others, only one determination was made on a subject of less than 37 or more than 50 kilos weight who had a marked acidosis without bicarbonate dosage. In this one, a boy of 12 in actual coma, both the original  $\sqrt{\frac{D}{W}} \sqrt{C}$  and the  $5\sqrt{\frac{D}{W}}$  formula

or of the sugar which accompanies them, apparently acts as a diuretic, and secures the necessary excretion. For example, Magnus-Levy reports that when a patient on 2 successive days excreted 109.5 and 157 gm. of organic acid calculated as hydroxybutyric, the corresponding urine volumes were 8.0 and 9.2 liters (Magnus-Levy (4), p. 182). Similar high excretions are seen in the data of Papers IV and VI when acid excretion, as indicated by the ammonia plus titratable acid, was high.

indicate a plasma  $\text{CO}_2$  of 30 per cent, which is a severe acidosis, though not so severe as that shown both by clinical condition and plasma  $\text{CO}_2$  of 14 per cent. If the body weight were neglected in the calculation, however, and Barnett's  $0.7 \sqrt{D}$  formula used, the excretion would indicate a plasma  $\text{CO}_2$  of 46, or almost no acidosis. Similar magnification of error is introduced in attempting to interpret the excretion data regardless of body weight in the two patients of less than adult size with acidosis reported in Paper VI. These are No. 3, a boy of 12 with intense acidosis, and No. 5, a boy of 13 with severe acidosis. That an allowance for body size must be made in interpreting the rate of formation or excretion of any metabolic product is a generalization so well founded that previous discussion of it seemed unnecessary.

Elimination of a third variable, the titratable acid, from the calculation, would apparently be a further step backwards. It is true, as exemplified by our own data, that ammonia and titratable acid in diabetic urine as a rule rise and fall together, the ammonia being usually two or three times the titratable acid. The ratio  $\frac{0.1 \text{ N NH}_3}{0.1 \text{ N acid}}$  is by no means constant, however, varying from 0.3 to 5.0 in diabetic and normal urines, so that the titratable acid sometimes exceeds the ammonia. Since both ammonia and titratable acid indicate excretion of acid in excess of fixed base, it does not seem logical to determine the one and neglect the other. The result of neglecting the titratable acid is apparent, except in the case of the one diabetic who was chosen for continuous observation, in a decided increase in the average error.

*Average Errors. Data of Paper IV.*

Formula	Table I. 11 normal persons.	Table II. 36 different diabetics.	Table III. 29 observations on 1 diabetic.
$\text{CO}_2 = 80 - 5\sqrt{\frac{D}{W}}$ .....	2.5	5.4	5.7
$\text{CO}_2 = 80 - 0.9\sqrt{\text{NH}_3}$ .....	4.6	7.4	5.7

A practical additional reason for determining titratable acid as well as ammonia is that it protects against a false diagnosis of acidosis

which might be made from the ammonia alone in urines that have undergone bacterial decomposition, either in the bladder as the result of cystitis, or outside the bladder as the result of preservation with insufficient antiseptis. As long as the ammonia formation leaves the urine still acid, it does not much alter the  $\text{NH}_3 + \text{acid}$  figure, since  $\text{CO}_2$  of the ammonium carbonate formed by bacterial action on urea, escapes, while the ammonia remains and neutralizes approximately an equivalent of acid. The net effect of an increase of ammonia is therefore an approximately equal decrease of titratable acid, with no significant influence on the resultant sum of the two.

Our data indicate furthermore that the ratio  $\frac{0.1 \text{ N } \text{NH}_3}{0.1 \text{ N } \text{acid}}$  may be used as a fairly sensitive indicator of ammoniacal decomposition. In the urines of Paper IV, which were all analyzed while perfectly fresh, the ratio varies from 2.3 to 4.8 averaging 1.6 in normal men and 2.3 in diabetics. In only one case was a value of 4.1 exceeded. It appears therefore that when the ammonia exceeds four or five times the titratable acid there is ground for suspecting the origin of a measurable portion of the ammonia in bacterial action. Although the 24 hour urines reported in Paper VI are believed to have been collected with at least ordinary care and were preserved with toluene, it will be seen from the results that in these urines the ammonia:acid ratio averages higher (about 4.1) than in the quickly collected and analyzed specimens of Paper IV, and frequently exceeds the maximum of the short-time urines, at times rising as high as 8 or 9. In no case had deposition gone so far as to neutralize all or nearly all of the titratable acid, so that the results, based on the sum of ammonia and titratable acid, could not have been significantly affected. The frequency of high ammonia:acid ratios in the 24 hour urines nevertheless indicates the readiness with which decomposition may occur in 24 hour specimens even when collected with routine precaution.

For the above reasons we believe that when diabetic acidosis must be estimated from acid excretion, the most satisfactory formula at present available for expressing the results in terms of alkaline reserve is



$$\text{Plasma CO}_2 = 80 - 5 \sqrt{\frac{0.1 \text{ N (acid + NH}_3\text{) per 24 hours}}{\text{kilos body weight}}}$$

and that neither the titratable acid nor body weight may be neglected without increasing the chance of error in the estimation.

As, thanks to Barnett, the acid excretion formula is simplified to the above by elimination of one unnecessary variable, and as Palmer and Van Slyke in Paper IX (5) have published data which add the bicarbonate retention to the indirect acidosis tests that have been composed with the direct, it appears desirable to revise accordingly the summarizing table in Paper VI (*Studies XXVIII*, p. 442).<sup>2</sup> In the following table we have therefore substituted the simpler expression for acid excretion and have added the data for the bicarbonate retention test. We have also indicated the chief fallacies to which, according to the results published in the present series of papers, each indirect test is liable when applied to the detection of diabetic acidosis.

It should be noted that the data obtained from kidney and lung excretion as measures of alkaline reserve in diabetic acidosis do not necessarily hold for other types of acidosis. In nephritis, for example, the two tests based on kidney excretion become fallacious, while Peters (6) has recently shown that in cardiac dyspnea and in conditions involving great diminution of lung capacity the mechanics of respiration are so disturbed that the alveolar carbon dioxide ceases to be an approximate measure of blood bicarbonate. The indirect tests may be trusted as approximate indicators of alkaline reserve only in conditions where they have been previously tested by comparison with the blood bicarbonate. We have made this comparison in diabetics, but the results do not hold for other pathological conditions.

#### SUMMARY.

Acid excretion as a measure of diabetic acidosis is, according to present data, most significantly expressed in terms of ammonia plus titratable acid per unit of body weight. The average error involved in estimating alkaline reserve from acid excretion is, as shown by Barnett, appreciably reduced by simplifying our original empirical formula,  $\text{plasma CO}_2 = 80 - \sqrt{\frac{D}{W}} \sqrt{C}$ , to  $\text{plasma CO}_2 = 80 - 5 \sqrt{\frac{D}{W}}$ .

<sup>2</sup> Also *J. Biol. Chem.*, 1917, xxx, 412.

Corresponding results of indirect tests for acidosis.

Condition of subject.	Actual bicarbonate reserve. Plasma bicarbonate CO <sub>2</sub> reduced to 0-7,760 mm.	24 hour excretion* of 0.1N acid + NH <sub>4</sub> .		Carbon dioxide of alveolar air.		Sodium bicarbonate required to turn urine alkaline.	
		(a) Cc. per kg. (b) Approximate cc. per 60 kg. person.	Reliability in diabetes.	(a) Mm. tension. (b) Approximate per cent.	Reliability in diabetes.	(a) Gm. per kg.† (b) Approximate gm. per 60 kg. person.	Reliability in diabetes.
Normal resting adult. Extreme limits of bicarbonate reserve.	vol. per cent 80-53	(a) 0-27 (b) 0-1,600	Good.	(a) 53-35 mm. (b) 6.8-4.7 per cent.	May indicate some acidosis in its absence.	(a) 0-0.5 (b) 0-30	May indicate acidosis in its absence.
Mild acidosis, no pronounced symptoms.	53-40	(a) 27-65 (b) 1,600-4,000	Good.†	(a) 35-27 mm. (b) 4.7-3.6 per cent.	May indicate more acidosis than is present.	(a) 0.5-0.8 (b) 30-50	May indicate much more acidosis than is present.
Moderate to severe acidosis. Symptoms may be apparent.	40-30	(a) 65-100 (b) 4,000-6,000	Liable† to considerable error in either direction.	(a) 27-20 mm. (b) 3.6-2.7 per cent.	Good.	(a) 0.8-1.1 (b) 50-65	"
Severe acidosis. Symptoms of acid intoxication.	Below 30	(a) Over 100 (b) " 6,000	"	(a) Below 20 mm. (b) Below 2.7 per cent.	"	(a) Over 1.1 (b) Over 65	"

\* Measured either in 24 hour urine or on specimen from shorter period calculated to 24 hour basis.

† After bicarbonate administration likely to indicate more acidosis than is present.

‡ The figures tabulated in this column also indicate the doses of bicarbonate necessary to restore the alkaline reserve to normal from acidosis of the severity indicated by the corresponding plasma CO<sub>2</sub> figures in the first column.

The absolute difference in the results calculated by the two equations is, however, so small that the remarks on the range of error in such calculations made in Papers IV and VI hold with essentially equal force when the simplified formula is used. Further simplification, by neglecting the body weight or titratable acid as suggested by Barnett, decreases the accuracy of the estimation.

For practical purposes the acid excretion may, without going through the calculation of the formula, be interpreted directly into terms of clinical severity of acidosis, as indicated in the table; *e.g.*, an excretion exceeding 27 cc. of 0.1 N ammonia plus acid per kilo indicates acidosis, which usually becomes critical if the excretion approaches 100 cc. per kilo.

The relationships of the plasma bicarbonate to acid excretion, alkali retention, and alveolar carbon dioxide tension are summarized for reference in a table, wherein are also indicated the chief errors to which, according to the data of Papers IV, VI, and IX of this series, the three latter determinations are subject as measures of diabetic acidosis.

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## INTESTINAL INTOXICATION IN INFANTS.

### THE IMPORTANCE OF IMPAIRED RENAL FUNCTION.\*

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Preceding the development of toxic symptoms in intestinal intoxication<sup>1</sup> there is pronounced diarrhea, vomiting and diminished ingestion of fluid, the relative importance of which varies in different cases. In the acute stage of the symptoms the excretion of urine is greatly diminished, and at this time the urine contains albumin and abundant casts.

During the past year investigation of cases of intestinal intoxication has furnished evidence which emphasizes the importance of the loss of fluid and the impaired secretion of urine. In this communication it is proposed to present such evidence and to discuss its significance with special reference to the symptomatology.

#### 1. *Nonprotein Nitrogen and Urea of the Blood.*

*Technic.*<sup>2</sup>—The nonprotein nitrogen was determined by the method of Gettler and Baker<sup>3</sup> except that the final determination was by aeration instead of by distillation. Fiftieth-normal acid and hundredth-normal alkali were used with

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1. It is realized that the use of the term "intestinal intoxication" is open to objections. This term is not applied to the complex described by Finkelstein as "alimentäre intoxication" but is used merely to designate the series of toxic symptoms which frequently occur in association with profound nutritional disorders in infants.

The term "diarrhea with toxic symptoms" is cumbersome and is not more exact, for there is not always a direct relation between the severity of the diarrhea and the degree of toxemia. Indeed, there are cases with symptoms indistinguishable from those of intestinal intoxication in which there is no diarrhea at all.

2. All blood for analysis was withdrawn from the superior longitudinal sinus by means of a syringe and the type of needle described in a previous communication. The blood was always taken at least three hours after the last meal.

3. Gettler, A. O., and Baker, W.: Jour. Biol. Chem., 1916, 25, 211.

methyl red as indicator. It was found that the presence of mercury used in the precipitation of the protein interfered with the aeration of ammonia, probably through the formation of a stable ammonia-mercury compound which was not broken up by alkali. At Dr. Gettler's suggestion potassium hydrogen sulphid was used to precipitate the mercury, and by this means the obstacle was overcome. A very strong pump was used for aeration and blank determinations demonstrated that aeration for one half hour was sufficient. This was shown, (1) by determinations of pure ammonium sulphate or urea, and (2) by distilling mixtures which had been aerated from ten to thirty minutes to determine whether all ammonia had been removed.

As a rule 3 c.c. of blood were used. In some cases in which the nonprotein nitrogen was increased, the determination was made with only 2 c.c. In the earlier cases all determinations were made in duplicate. These were always within the limits of experimental error, and owing to the limited amount of blood one can take from infants, duplicates were omitted in the later cases. The purity of all reagents was checked by blank determinations. Whenever ammonia was present the amount was regularly deducted.

Urea was determined by the urease method of Van Slyke and Cullen.<sup>4</sup> The enzyme was tested for ureolytic power and also to exclude the presence of ammonia. Fiftieth-normal acid and hundredth-normal alkali were used with methyl red as indicator.

*Normal Infants.*—Determinations were made on thirty normal infants. Such infants were of practically normal weight, had normal appetite and bowel movements and were apparently well. The values for urea and nonprotein nitrogen are shown in Table 1.

In such infants the nonprotein nitrogen ranged from 23 to 39.1 mg. per 100 c.c. of blood. These figures are considerably higher than those obtained by Minsk and Sauer,<sup>5</sup> who used Folin's method. The difference is probably due to the difference in methods, as the values for normal adults obtained by Gettler and Baker are higher than those given by Folin's method.

The urea nitrogen was from 4.9 to 14.6 mg. per 100 c.c. of blood. In only a few cases was the urea nitrogen approximately one half of the nonprotein nitrogen, which is generally the case in adults and is what Minsk and Sauer<sup>5</sup> found in their patients. The difference in these results and those of Minsk and Sauer is due mainly to the fact that the values for nonprotein nitrogen were greater than theirs and not to the difference in the amounts of urea nitrogen.

4. Van Slyke, D. D., and Cullen, G. E.: Jour. Am. Med. Assn., 1914, 62, 1558.

5. Minsk, L. D., and Sauer, L. W.: AM. JOUR. DIS. CHILD., 1917, 13, 397.

TABLE 1.

*The Nonprotein Nitrogen and Urea Nitrogen in the Blood of Normal Infants.\**

Number	Age, Months	Weight		Carbon Dioxide Tension, Alveolar Air, Mm.	Nonprotein Nitrogen, Mg. per 100 C.c. of Blood	Urea Nitrogen, Mg. per 100 C.c. of Blood
		Lb.	Oz.			
1	1½	9	4	38.2	33.35	8.6
2	1½	9	1	36.6	35.1	7.4
3	1½	9	2	41.2	30.15	13.8
4	2½	10	1	39.1	34.0	12.9
5	2½	10	4	42.4	35.2	10.3
6	3	11	4	40.6	37.6	4.9
7	3½	11	4	37.6	39.1	7.7
8	3½	11	8	35.9	36.2	13.3
9	3½	11	4	38.7	38.0	11.8
10	4	12		41.2	39.1	10.9
11	4½	12	4	40.1	35.2	12.2
12	4½	11	12	36.1	35.4	12.3
13	4½	13	12	40.0	39.0	11.3
14	5½	14	1	39.1	27.6	8.3
15	5½	13	4	36.8	29.8	11.2
16	5½	12	15	40.1	38.2	7.1
17	5½	14	14	44.6	26.6	8.3
18	6	15	8	37.8	27.4	14.6
19	6½	15	14	43.2	34.3	13.9
20	7	18	4	35.8	31.1	7.9
21	7½	17	2	39.2	38.0	11.8
22	7½	16	12	40.3	24.2	11.7
23	7½	17	4	41.2	23.0	7.8
24	8½	17	12	36.7	31.6	10.7
25	9	18		38.9	27.5	4.9
26	9½	18	14	37.6	25.0	10.2
27	10	17	14	42.3	38.4	11.3
28	11	21	11	36.4	34.3	10.1
29	11½	20	4	40.1	31.2	7.2
30	12	21	4	37.5	28.0	9.3

\* The air was collected by the Plesch-Higgins method as modified by Howland and Marriott. The air analyses were made in the Haldane apparatus.

As shown in Table 1, the amount of urea nitrogen varies greatly in different infants. A careful analysis of the food and the method of feeding fails to reveal an adequate cause for this variation. It seems possible that such variation may be due to the different rate of urea elimination by the kidney in accordance with Ambard's hypothesis.

*Infants with Intestinal Intoxication.*—Forty-six patients with intes-

TABLE 2.

*The Nonprotein Nitrogen and Urea Nitrogen in the Blood of Infants with Intestinal Intoxication.\**

Number	Date	Age, Months	Weight		Carbon Dioxid Tension of Alveolar Air, Mm.	C.c. of CO <sub>2</sub> , Reduced to 0.760 Mm., Bound as Bicarbonate by 100 C.c. of Blood Plasma (Van Slyke)	Nonprotein Nitrogen, Mg. per 100 C.c. of Blood	Urea Nitrogen, Mg. per 100 C.c. of Blood
			Lb.	Os.				
1	1/12	3	8	15	22.0	40.0†	140.0	62.3
	1/14‡				42.0	92.0	98.0	
	1/18				33.0		29.0	11.1
	1/24					65.0	28.0	
2	1/15	5	10		19.0	....	58.0	20.1
	1/16				20.0	36.0	123.0	
3	2/ 2	5½	12	4	14.7§	26.0	140.0	
	2/ 5				17.4§		193.2	
	2/ 6					34.0	184.8	
4	3/ 2	4	6	6	31.37§	....	137.0	
	3/ 3				21.0	42.0	193.0	
5	3/ 5	3	8	8	17.0	....	150.0	84.0
	3/ 6					38.0	169.0	75.6
6	3/10	5½	5	9	34.2§	....	83.0	39.0
	3/12					40.0	73.0	
	3/13				22.0		97.0	
7	3/19	4	13	6	35.1§	....	78.6	25.2
	3/20				17.2§			
	3/21					88.0		
	3/26‡				36.4§	100.0	57.0	15.9
8	4/18	9	17		28.4§	....	142.0	72.1
	4/19‡				56.0	97.0	127.0	64.3
	4/26					69.0	95.0	
9	.....	6	11	4	21.5§	40.0	84.0	39.7
10	.....	11½	12		17.0	....	61.9	
11	.....	13	15	3	23.4§	25.1	124.0	44.8
12	.....	6	12	8	25.0	....	133.0	39.1
13	5/16	5	11	12	23.0	....	134.4	
	5/17‡				37.5§	76.0	176.9	76.58

TABLE 2.—*Continued.*

Number	Date	Age, Months	Weight		Carbon Dioxid Tension of Alveola Air, Mm.	C.c. of CO <sub>2</sub> Reduced to 0.750 Mm., Bound as Bicarbonate by 100 C.c. of Blood Plasma (Van Slyke)	Nonprotein Nitrogen, Mg. per 100 C.c. of Blood	Urea Nitrogen, Mg. per 100 C.c. of Blood.
			Lb.	Oz.				
14	.....	8½	14	3	17.0	....	232.0	108.2
15	.....	5	10	4	23.6§	....	179.0	68.4
16	.....	7	13	1	26.1§	....	58.8	27.6
17	.....	8½	14	2	16.0	34.0	143.0	78.7
18	.....	9½	13	4	16.0	....	36.0	13.2
19	11/11	7	11	6	21.3§	....	176.0	89.0
	11/18†				38.0		160.0	
	11/24						28.0	7.0
20	5/20	9	14	8	14.4§	23.2	211.0	96.1
	5/25‡				44.3§		50.1	13.4
21	5/23	9	14	4	24.6§	....	189.0	71.9
	5/27‡				39.6§		34.0	14.0
22	6/ 1	10	15	6	23.0	....	140.0	59.7
23	.....	13	19		25.0	....	159.6	63.2

\* This table includes only part of the cases, as forty-four cases of intestinal intoxication were investigated. The results were so uniform that it seemed superfluous to tabulate all cases. Some of the additional cases will be cited in the text.

† These values are cited merely in general confirmation of the alveolar air analyses. They cannot be compared accurately with the figures for alveolar air, since the values for the carbon dioxide of alveolar air represent venous blood while the plasma analyses are probably more nearly representative of arterial blood. In a late series of cases, to be reported shortly, this discrepancy has been eliminated.

‡ After treatment. The treatment comprised the administration of 2 to 7 gm. of sodium bicarbonate by subcutaneous or intravenous injection and giving large amounts of water by mouth, or salt solution subcutaneously or intravenously.

§ Air collected by the method of Plesch-Higgins as adapted to infants by Howland and Marriott and analyzed in the Haldane apparatus. The remaining figures represent alveolar air collected in the same way but determined by the colorimetric method of Marriott.



tinal intoxication were studied. Thirty-six had acidosis and thirty-three of these showed an increase in the nonprotein nitrogen of the blood. There were, therefore, only three patients with acidosis without an increase of the nonprotein nitrogen. In only one of these patients was more than one determination made. There were four cases without acidosis but with an increased nonprotein nitrogen and four cases with neither acidosis nor increased nonprotein nitrogen. Thirty-seven of the forty-six cases showed a decided increase in the nonprotein nitrogen of the blood.

Twenty-three of the cases with acidosis are shown in Table 2. These were selected for presentation as they were the ones most completely observed, and, moreover, they are representative of the entire series. Of these cases there was only one with no increase in the nonprotein nitrogen (Case 18). In the remainder the nonprotein nitrogen ranged from 57 to 232 mg. per 100 c.c. of blood, the urea nitrogen from 20.1 mg. to 108.2 mg. per 100 c.c. of blood. Comparison with the results from normal infants (Table 1) shows that in intestinal intoxication the urea and nonprotein nitrogen are much increased.

Minsk and Sauer<sup>5</sup> determined the nonprotein and urea nitrogen in six cases of intestinal intoxication; in three there was a distinct increase. The greatest nonprotein nitrogen in their cases was 67.6 mg. nitrogen per 100 cc. of blood, which was much lower than in most of my cases. The urea nitrogen in their cases constituted a larger percentage of the nonprotein nitrogen than in my cases, but the actual values for urea nitrogen obtained by them were less.

*Discussion.*—It is therefore apparent that infants suffering from intestinal intoxication show a marked increase of nonprotein nitrogen and urea in the blood. The nonprotein nitrogen of the blood consists of urea, uric acid, creatin, creatinin, ammonia, amino-acids and probably other substances of unknown nature. As shown in Table 2, the increase in nonprotein nitrogen is due in part only to urea, which comprises less than 50 per cent. of the total nonprotein nitrogen. Determinations of the amounts of uric acid, creatinin, ammonia and amino-acids in this series of cases would have been of interest and perhaps of practical importance, but they were not made, as more blood would have been required than we felt justified in taking. Determinations of these substances, however, have been made in individual cases and are being concluded at the time of preparing this article.

In three cases of the present series uric acid and creatinin were determined and a decided increase found. The results were as follows:

Case	Nonprotein N, Mg. per 100 C.c. Blood	Urea N, Mg. per 100 C.c. Blood	Uric Acid, Mg. per 100 C.c. Blood	Creatinin, Mg. per 100 C.c. Blood
21	189.0	71.9	18	3.0
22	140.0	59.7	21	2.1
23	159.6	63.2	10	2.9

The substances comprising the nonprotein nitrogen are normally excreted in the urine, and an accumulation in the blood usually signifies an impairment of renal function. While this interpretation is generally accepted, yet in connection with infantile diarrhea another possibility must be considered. The loss of water through the bowels in diarrhea is one of the most prominent clinical features of the disease and the question naturally arises whether this loss of water could be responsible for the high nonprotein nitrogen in the blood. If the blood became more concentrated through loss of water, it seems possible that there could be a relative increase of nonprotein nitrogen due to this cause. Such an increase, however, would be merely apparent.

To obtain evidence bearing on this possibility, determinations of the blood concentration were made.

## *II. The Concentration of the Blood.*

*1. Corpuscular Volume.*—This was determined in the special pipet of Gettler and Baker<sup>3</sup> and in the manner described by them.

The corpuscular volume of normal infants ranges from 31 to 42 volumes per cent. (Table 3). Gettler and Baker found in normal adults that the corpuscular volume ranged from 33 to 50 volumes per cent., which values are considerably higher than those in normal infants.

In cases of intestinal intoxication the values were from 32 to 49 volumes per cent. (Table 4). Comparison of Tables 3 and 4 shows that in cases of intestinal intoxication the average corpuscular volume is higher, and in a number of the individual cases this increase is marked. The corpuscular volume, however, is not regularly increased in the cases of intoxication, and in a number of instances, despite the increase in nonprotein nitrogen, the corpuscular volume was within

normal limits. Of most significance was the fact that there was no direct ratio between the corpuscular volume and the nonprotein nitrogen. For example, Case 5 showed a corpuscular volume of 38 and a

TABLE 3.

*The Corpuscular Volume and Total Solids of the Blood; the Specific Gravity and Total Nitrogen of the Blood Serum of Normal Infants.\**

Number	Total Solids of the Blood, per Cent.	Corpuscular Volume of the Blood, per Cent.	Specific Gravity of the Blood Serum	Total Nitrogen of the Blood Serum, Gm. per 100 C.c.	Ratio of Nonprotein Nitrogen of Blood to Total Nitrogen of the Blood Serum N.P.N. : T.N.
1	....	25.2	1.021	1.123	1 : 34
2	....	34.3	1.026	1.088	1 : 31
3	....	38.9	1.024	1.101	1 : 37
4	....	42.0	1.023	0.9876	1 : 29
5	....	38.0	1.021	1.043	1 : 30
6	....	32.5	1.020	1.181	1 : 32
7	....	35.5	1.022	1.198	1 : 31
8	....	34.0	1.020	1.11	1 : 31
9	....	33.0	1.025	1.02	1 : 27
10	....	29.0	1.024	0.983	1 : 25
11	....	34.0	1.025	1.13	1 : 32
12	....	31.0	1.023	0.987	1 : 28
13	18.6	45.0	1.022	1.061	1 : 27
14	17.9	39.0	1.020	1.113	1 : 40
15	....	32.0	1.021	0.997	1 : 33
16	17.5	33.0	1.022	1.043	1 : 28
17	17.1	29.8	1.024	1.121	1 : 41
18	....	32.0	1.026	1.069	1 : 40
19	17.7	36.0	1.020	1.00	1 : 29
20	....	37.0	1.026	0.983	1 : 32
21	....	33.0	1.025	1.18	1 : 31
22	18.4	38.0	1.020	1.108	1 : 46
23	17.3	31.0	1.024	1.16	1 : 50
24	18.2	39.0	1.023	0.977	1 : 30
25	17.2	32.9	1.021	1.111	1 : 40
26	....	34.0	1.020	1.023	1 : 41
27	....	32.0	1.021	1.101	1 : 29
28	17.4	34.0	1.022	1.097	1 : 32
29	....	35.0	1.023	1.114	1 : 36
30	18.5	39.8	1.025	1.01	1 : 36

\* The figures in this table were obtained from the same infants as those in Table 1 and are numbered the same. To avoid needless repetition the age, weight, etc., are not given in the foregoing table as they are shown in Table 1.

TABLE 4.

*The Corpuscular Volume and Total Solids of the Blood; the Specific Gravity and Total Nitrogen of the Blood Serum of Infants with Intestinal Intoxication.\**

Number	Date	Total Solids of the Blood, per Cent.	Corpuscular Volume of the Blood, per Cent.	Specific Gravity of the Blood Serum	Total Nitrogen of the Blood Serum, Gm. per 100 c.c.	Ratio of the Nonprotein Nitrogen of the Blood to the Total Nitrogen of the Blood Serum N.P.N. : T.N.
1	1/12	....	36.0	1.0279	1.397	1 : 10
	1/14		37.0	1.025	1.36	1 : 14
	1/18		32.0	1.023	1.11	1 : 38†
2	1/15	....	39.5	1.026	—	
	1/16	21.8	—	1.027	1.55	1 : 13
3	2/ 2	....	47.0	1.029	1.36	1 : 10
	2/ 6	20.4	34.0	1.029	1.219	1 : 6
4	3/ 2	....	32.0	—	—	
	3/ 3	20.9	31.0	1.030	1.368	1 : 7
5	3/ 5	20.6	38.0	1.031	—	
	3/ 6	19.7	30.0	1.027	1.112	1 : 7
6	3/10	21.1	—	1.026	1.114	1 : 13
	3/12	20.0	—	—	0.914	1 : 12
	3/13		37.0	1.030	1.28	1 : 13
7	3/19	....	49.0	1.030	1.176	1 : 15
	3/21		39.0	—	—	
	3/26	20.8	35.0	1.024	0.985	1 : 17
8	4/18	21.6	37.0	1.032	1.513	1 : 11
	4/19	21.1	42.0	1.030	1.42	1 : 11
	4/26	19.3	27.0	1.026	0.942	1 : 10
9	....	....	—	—	—	
10	....	....	35.0	1.024	1.057	1 : 17
11	....	....	39.0	1.030	—	
12	....	20.8	49.0	1.028	1.218	1 : 9
13	5/16	21.3	42.0	1.029	1.358	1 : 10
	5/17		37.0	1.026	1.204	1 : 7
14	....	21.1	49.0	.....	1.36	1 : 6

TABLE 4—*Continued.*

Number	Date	Total Solids of the Blood, per Cent.	Corpuscular Volume of the Blood, per Cent.	Specific Gravity of the Blood Serum	Total Nitrogen of the Blood Serum, Gm. per. 100 c.c.	Ratio of the Nonprotein Nitrogen of the Blood to the Total Nitrogen of the Blood Serum N.P.N. : T.N.
15	....	....	....	1.030	1.302	1 : 7
16	....	17.4	....	1.023	0.980	1 : 17
17	....	....	47.0	1.029		
18	....	....	29.0	1.026		
19	11/11	19.9	39.6	1.030	1.3	1 : 7
	11/18		38.4		1.23	1 : 8
	11/24	18.9†	34.0	1.027	1.09	1 : 39†
20	5/20	21.7	39.8	1.031	1.365	1 : 6
	5/25	19.1†	37.0	1.025	1.071	1 : 22†
21	5/23	21.6	45.0	1.029	1.361	1 : 7
	5/27	18.5†	38.9	1.023	1.14	1 : 33†
22	....	....	49.9	1.030	1.293	1 : 9
23	....	....	46.2	1.031	1.321	1 : 8

\* The figures in this table were obtained from the same infants as those in Table 2 and are numbered the same. To avoid needless repetition the age; weight, etc., are not given in the above table as they are in Table 2. See footnotes \* and † Table 2.

† After treatment.

nonprotein nitrogen of 150 mg. per 100 c.c. of blood, and later a corpuscular volume of 30 and a nonprotein nitrogen of 169. In both instances the corpuscular volume is within normal limits while the nonprotein nitrogen is greatly increased. Moreover, the second determination showed a lower corpuscular volume in the presence of a slightly increased nonprotein nitrogen. The same evidence is shown in several of the tabulated cases.

These determinations show that there is no constant decrease of the blood plasma in relation to corpuscles and that according to these results the high nonprotein nitrogen in cases of intestinal intoxication cannot be explained on the basis of increased blood concentration.

This argument would be valid only if, in intestinal intoxication, there was no increased destruction of red cells and if the red cells were of normal size. Obviously, a marked destruction of red cells would decrease the corpuscular volume while an increase or decrease in the size of the cells would be of marked influence. Whether there is an increased blood destruction in intestinal intoxication is impossible to say, but there is no evidence to support such a view. Fresh blood preparations have been examined in a number of cases and these showed no apparent change in the size of the red cells.

2. *Specific Gravity of the Blood Serum.*—The specific gravity was determined by weighing the blood serum in small Nicholl pyknometers with a capacity of from 0.4 to 1.5 gm. of water. All determinations were at 22 C.

The specific gravity of the blood serum of normal infants varies from 1.020 to 1.025 (Table 3). These values are lower than those in normal adults, which, according to Gettler and Baker,<sup>3</sup> are from 1.026 to 1.030. In cases of intoxication the specific gravity was from 1.026 to 1.031, which is greater than that of normal infants. This increase, however, is too slight to account for the increase in nonprotein nitrogen merely on the basis of increased blood concentration.

Assuming that the normal specific gravity of the blood serum of infants is 1.020, this signifies that each 100 c.c. of blood serum contains 2 gm. of dissolved solids. An increase of the specific gravity to 1.031, which was the maximum found in cases of intoxication, would indicate an increase of 1.1 gm. of solids per 100 c.c. This would merely signify that the serum contained a trifle more than one and one-half times as much solids as normal. In the cases of intestinal intoxication, we find that the nonprotein nitrogen is often five times as great as normal, and if this increase were merely a matter of increased blood concentration, all solids should be equally affected and the specific gravity should be as great as 1.100. In no instance was the specific gravity increased to any degree comparable to the increase in nonprotein nitrogen. Moreover, a comparison of the nonprotein nitrogen and specific gravity in cases of intoxication shows that there is no direct ratio and that in several instances there was a great increase in the nonprotein nitrogen, with a specific gravity within the limits of normal. Moreover, the mere increase in nonprotein nitrogen would cause an increase in the

specific gravity of the serum aside from any increase in concentration due to loss of fluid from the blood.

3. *Total Nitrogen of the Blood Serum.*—One half to 1 c.c. of blood serum was digested in a small Kjeldahl flask with 2 c.c. of sulphuric acid, about 0.5 gm. of potassium sulphate and a small crystal of copper sulphate. The determination was made by aeration into twentieth-normal acid and titration with twentieth-normal sodium hydroxid, using congo red as indicator.

If the increase of nonprotein nitrogen in the blood of infants suffering from intestinal intoxication is due merely to increased concentration of the blood, there should be a similar increase in the other substances comprising the total nitrogen of the blood. That such is not the case is readily seen by referring to Tables 3 and 4.<sup>6</sup> While there is a greater total nitrogen of the blood serum in cases of intoxication, this increase is in no way comparable to the increase of nonprotein nitrogen, and in several cases the increase in total nitrogen can be explained almost entirely by the increase in nonprotein nitrogen. This is shown best by comparing the nonprotein nitrogen: total nitrogen ratio in the normal cases with those of intoxication.

4. *Total Solids of the Blood.*—The total solids were determined by weighing a few drops of blood on blotting paper previously weighed, drying at 110 C. and again weighing.

As shown in Table 3, the total solids in normal infants ranged from 17.1 to 18.6 per cent. In cases of intoxication (Table 4) the values were from 19.6 to 21.8 per cent. These figures agree closely with those recently published by Courtney and Fales<sup>7</sup> and have the same significance as those for corpuscular volume, total nitrogen and specific gravity.

*Discussion.*—It is of interest that the corpuscular volume of the blood, the total solids of the blood, the total nitrogen and specific gravity of the blood serum are all much lower in normal infants than in adults. This is probably in correspondence with the fact that the

6. In adults Gettler and Baker (Footnote 3) found that the total nitrogen of the blood serum was from 1.2 to 1.4 gm. per 100 c. c. These values are much greater than those from normal infants.

7. Courtney, A. M., and Fales, H. L.: *AM. JOUR. DIS. CHILD.*, 1917, 14, 202.

tissues of the infant contain a higher percentage of water and that the water metabolism is more active in infants than in adults.

The data furnished by the total nitrogen, specific gravity, corpuscular volume and total solids indicate that in intestinal intoxication there is often an increase in blood concentration. This increase in concentration, however, is insufficient to account for the increase in non-protein nitrogen found in these cases.

Further evidence bearing on this question is the observation that a number of patients begin to retain fluid for some time before the non-protein nitrogen falls to normal. The nonprotein nitrogen remains high after the corpuscular volume of the blood and total nitrogen and specific gravity of the serum reach normal. This would indicate that the high nonprotein nitrogen was not due merely to the increased blood concentration. The results from a case of this kind are shown in Table 5.

TABLE 5.

*Showing the Retention of Nonprotein Nitrogen and Urea After the Blood Concentration Became Normal.*

Date	Weight, Pounds	Nonprotein Nitrogen, Mg. per 100 C.c. of Blood	Urea Nitrogen, Mg. per 100 C.c. of Blood	Specific Gravity of Blood Serum	Total Nitrogen in Blood Serum, Gm. per 100 C.c.	Corpuscular Volume, per Cent.
11/11	11 $\frac{1}{2}$	176	89	1.030	1.301	39.6
11/18	11	160	..	.....	1.23	38.4
11/20	11 $\frac{1}{2}$	120	50	1.026	1.18	34.5
11/22	11 $\frac{1}{2}$	100	..	1.025	1.12	
11/24	11 $\frac{1}{2}$	28	7	1.024	1.09	34.0

Baby G. (Case 19) was admitted Nov. 11, 1916, with a history of severe diarrhea and vomiting for four days. The infant was greatly emaciated and the fontanel was sunken. The mental condition was dull. Hyperpnea was marked and the carbon dioxid tension of the alveolar air was 19 mm.

Seven and a half grams of sodium bicarbonate were given by intravenous injection. The carbon dioxid tension of the alveolar air became normal and the hyperpnea ceased. Large amounts of water were given by mouth and by subcutaneous injection (physiologic sodium chlorid solution).

On the basis of the evidence given it seems that the increased non-protein nitrogen which occurs in cases of intestinal intoxication is due to defective elimination of the nitrogenous waste products by the kidney.



### III. Tests of Renal Function. Phenolsulphonephthalein Elimination: Ambard's Coefficient of Urea Excretion.

*Phenolsulphonephthalein Elimination: Technic.*—The infant was put on a metabolism frame three hours after the last feeding and was given 120 c.c. of slightly sweetened water. Three mg. of phenolsulphonephthalein were given intramuscularly and the urine collected for two hours. One half of the urine was used to determine the phthalein in a Rowntree and Geraghty modification of the Autenreith-Königsberger colorimeter; the other half was used for estimation of urea, ammonia and total nitrogen.

Tests were made on thirty normal infants from 3 months to 1 year of age. The phenolsulphonephthalein elimination ranged from 58 to 94 per cent. during two hours, the water elimination from 56 to 100 c.c. (47.5 to 83.5 per cent.). (Table 6.)

TABLE 6.

*The Elimination of Water and Phenolsulphonephthalein; Ambard's Coefficient of Urea Excretion. Normal Infants.*

Number	Age, Months	Weight, Pounds	Water Elimination after Ingestion of 120 C.c. of Water, 2 Hour Period		Phenolsulpho- nephthalein Output, 2 Hour Period, per Cent.	Ambard's Coefficient of Urea Excretion, 2 Hour Period, Ur $\sqrt{D \frac{\sqrt{c}}{\sqrt{5}}}$
			C.c.	per Cent.		
1	3	11½	66	55.0	80	0.072
2	3½	10½	80	67.5	64	0.061
3	4	12½	90	75.0	68	0.096
4	4	11½	58	47.5	96	0.084
5	5	13	74	61.5	58	0.10
6	5½	13½	94	78.0	73	0.071
7	6	14½	59	49.0	67	0.05
8	6	15	67	56.0	74	0.11
9	6½	14½	86	71.5	88	0.065
10	7	16½	73	61.0	71	0.093
11	7	16	81	62.5	81	0.061
12	7½	15½	59	49.0	61	0.01
13	8	16½	62	52.0	70	0.072
14	8	17½	56	47.5	59	0.076
15	10½	18	79	66.0	86	0.068
16	11	19½	100	83.5	94	0.05
17	12	20½	77	64.0	87	0.078
18	13	21	67	56.0	75	0.087
19	13	20½	99	82.5	71	0.061
20	13½	21½	78	65.0	78	0.072

The results from 16 infants with intoxication are shown in Table 7. The phenolsulphonephthalein elimination was from 14 to 38 per cent., the water elimination from 23 to 48 c.c. (19 to 40 per cent.). The interpretation of these results in terms of renal permeability cannot be made with any degree of assurance, owing to the greatly diminished

TABLE 7.

*The Elimination of Water and Phenolsulphonephthalein; Ambard's Coefficient of Urea Excretion. Infants with Intestinal Intoxication.\**

Number	Age, Months.	Weight, Pounds	Water Elimination after Ingestion of 120 C.c. of Water, 2 Hour Period		Phenol-sulphonephthalein Output, 2 Hour Period, per Cent.	Ambard's Coefficient of Urea Excretion, 2 Hour Period, $\frac{U_r}{\sqrt{D} \sqrt{\frac{c}{5}}}$
			C.c.	per Cent.		
1	3	8 $\frac{3}{4}$	26	13.0	22.0	0.23
2	4 $\frac{1}{2}$	9 $\frac{1}{4}$	31	26.0	18.0	0.31
3	5	9	40	33.0	29.0	0.21
4	5 $\frac{3}{4}$	10	25	31.0	34.0	0.19
5	6 $\frac{1}{2}$	9 $\frac{3}{4}$	48	40.0	34.0	0.22
6	7 $\frac{1}{2}$	12 $\frac{1}{4}$	35	29.0	17.0	0.36
7	7 $\frac{3}{4}$	11 $\frac{3}{4}$	29	24.0	20.0	0.27
8	8	13 $\frac{1}{2}$	31	26.0	23.0	0.29
9	8 $\frac{1}{2}$	12 $\frac{3}{4}$	23	19.0	15.5	0.27
10	9	14	47	40.0	38.0	0.10
11	9	13 $\frac{1}{2}$	36	30.0	27.0	0.17
12	10 $\frac{1}{2}$	12 $\frac{3}{4}$	28	23.0	14.0	0.37
13	10 $\frac{3}{4}$	13 $\frac{1}{4}$	30	25.0	30.0	0.20
14	11	14	37	31.0	29.0	0.27
15	11 $\frac{1}{2}$	12 $\frac{1}{4}$	44	37.0	39.0	0.18
16	11 $\frac{3}{4}$	15 $\frac{1}{4}$	33	27.5	20.0	0.23

\* During the acute stage of intestinal intoxication so little urine is secreted that tests of renal function are impossible. The figures shown were derived from a study of patients who had begun to improve and who excreted more than 20 c. c. of urine during the two-hour period.

secretion of urine. During the acute stage of intestinal intoxication the infant may pass no urine for hours and the twenty-four hour elimination may total only a few cubic centimeters. For this reason, in the cases of intestinal intoxication used for the phenolsulphonephthalein test the patients had recovered from the acute stage and

had commenced to pass moderate amounts of urine. No test was included during which less than 20 c.c. of urine was voided. Despite the selection of cases and the fact that 120 c.c. of water were ingested at the time of the test, the water output was greatly diminished. As shown by Tables 6 and 7, the phenolsulphonephthalein elimination was greatly diminished in cases of intoxication. Owing to the decreased secretion of urine the low phenolsulphonephthalein output cannot be attributed to an anatomic kidney lesion.

*Ambard's Coefficient of Urea Excretion.*—According to Ambard<sup>8</sup> and his co-workers there is a definite ratio between the urea in the circulating blood and that excreted in the urine per unit of time. The formula used to derive the coefficient is obtained from Ambard's third law and is as follows:

$$K = \frac{Ur}{\sqrt{D \frac{70 \sqrt{C}}{P \sqrt{25}}}}$$

K = Coefficient of urea excretion.

Ur = Urea per liter of blood.

D = Output of urea for twenty-four hours calculated from the amount excreted during the period of observation.

P = Weight of patient in kilograms.

C = Grams of urea per liter of urine.

70 = Standard weight.

25 = Normal amount of urea per liter of urine.

Our observations were made during two-hour periods at the same time the phenolsulphonephthalein tests were made. The analytical methods were those previously described.

In applying Ambard's formula to infants it is obvious that certain changes must be made. In the formula the standard weight of 70 kg. is used. This is, of course, relative to adults. It is practically impossible to introduce a standard weight for young infants owing to the

8. Ambard, L.: *Physiologie normale et pathologique des reins*, Paris, 1914; Ambard, L., and Weill, A.: *Jour. de physiol. et de pathol. gén.*, 1912, 32, 217. This subject has also been considered in detail by McLean, F. C., and Selling, L.: *Jour. Biol. Chem.*, 1914, 19, 31; McLean, F. C.: *Jour. Exper. Med.*, 1915, 22, 212; Addis, T., and Watanabe, C. K.: *Jour. Biol. Chem.*, 1916, 24, 203, and Lewis, D. S.: *Arch. Int. Med.*, 1917, 19, 1.

fact that the weight changes are so rapid. The use of the average weights for different ages would not be strictly accurate, since the weights of normal infants of the same ages may vary within several pounds, which makes a considerable error when one considers such differences in relation to total weight. A further difficulty arises from the circumstance that infants with nutritional disorders show great fluctuations in weight within short periods of time. Actual calculations in normal cases show that the coefficient is more constant if the factor  $70/P$  or any equivalent is eliminated.

For adults the standard value of 25 gm. of urea per liter of urine is used. Our determinations on thirty normal infants under 1 year of age gave figures which ranged from 4.2 to 6.37 gm. of urea per liter. On the basis of these figures the standard value of 5 was substituted for 25 in the formula. As used by us the formula was as follows:

$$K = \frac{U_r}{\sqrt{D \frac{\sqrt{C}}{\sqrt{5}}}}$$

In adults it has been found that the normal coefficient ranges from 0.6 to 0.9 and that defective kidney function causes the constant to increase. In normal infants the constant, as calculated by the formula we used, ranges from 0.05 to 0.11 (Table 6). The coefficient, therefore, has a tendency to be higher in infants than in adults, and in several of our normal cases values above 0.09 were obtained which in adults would signify impairment of renal function.

In cases of intoxication the constant ranged from 0.13 to 0.39, thus indicating a considerable degree of impairment of renal function (Table 7).

In general, these results are roughly parallel to the phenolsulphophthalein elimination and are open to the same criticism in reference to the excretion of water. All that these tests signify is that in intestinal intoxication the power of elimination by the urine is greatly impaired.

#### *IV. The Cause of Deficient Elimination by the Kidneys.*

In consideration of the greatly diminished excretion of urine during the acute stage of intestinal intoxication it seems only natural to expect that substances normally excreted in the urine would be retained.

This would certainly occur unless there were some other channel through which the waste products could be eliminated. Despite the self evidence of this proposition it is a question of some importance whether the retention is entirely a matter of lessened water excretion, or whether it is dependent on a kidney lesion.

The occurrence of albumin and casts in the urine of infants affected with severe diarrhea has long been recognized and is mentioned in practically every treatise on this disease. The subject was considered in detail by Pick,<sup>9</sup> in 1905, who gave an exhaustive review of the literature. He noted that the toxic symptoms in severe diarrhea are not unlike those of uremia, and suggested the possibility that in some cases the symptoms may be of uremic origin.

Neuman,<sup>10</sup> in 1907, found that in general there was a close parallel between the presence and degree of albuminuria and cylindruria and the severity of the toxic symptoms. He concluded, however, that the renal lesions were probably secondary and not of etiologic importance.

In all of the cases in this series the urine contained albumin and casts during the acute stage of the disease. With improvement in the symptoms, albumin and casts soon disappeared. The degree of albuminuria, however, was rarely great and not often of sufficient amount to give a reading in the Esbach tube. In most instances the number of casts seemed much greater than one would expect from the amount of albumin present. The period at which the albuminuria and cylindruria were most marked coincided with the period when the urine was very scanty, so that the number of casts, at least, would appear much greater than it actually was.

A detailed consideration of the significance of albumin and casts in the urine is beyond the scope of this paper. Certainly their presence is abnormal, but whether their occurrence indicates a kidney lesion capable of causing retention of nitrogenous waste products, is impossible to determine. It does seem clearly established, however, that the occurrence of albuminuria and cylindruria does not necessarily indicate a kidney lesion which is demonstrable by our present histologic methods. If definite anatomic lesions are the sole criteria

9. Pick, J.: Arch. f. Kinderh., 1905, 40, 291.

10. Neuman, G.: Jahrb. f. Kinderh., 1907, 66, 633.

of kidney disease, we should not be warranted in assuming that a kidney lesion was present solely on the basis of albuminuria and cylindruria.

Neuman<sup>10</sup> found slight parenchymatous renal lesions in one of his cases. In another, the kidneys were practically normal. Pick,<sup>9</sup> however, found distinct anatomic lesions in several cases. Definite renal lesions in infants dying of intestinal intoxication were described by Hoffsten,<sup>11</sup> Epstein,<sup>12</sup> Bernhard and Felsenthal,<sup>13</sup> Czerny and Moser,<sup>14</sup> Jehle<sup>15</sup> and others.

In nine of the cases presented in this paper necropsies were performed and the results are shown in Table 8. In two cases, 14 and H., there was distinct parenchymatous nephritis, severe in one (14) and moderate in the other (H.). In both of these cases the tubules contained many necrotic cells which were often massed to form distinct foci. These foci stained a deep blue with hematoxylin, which suggested the deposition of calcium salts. The lesions in these cases resembled closely those seen in poisoning with mercuric chlorid. Three cases, 10, 5 and O'D., showed no definite renal changes at all. The remaining four cases showed slight or moderate parenchymatous changes in the tubular epithelium and hemorrhage into the tubules. Dr. Charles Norris, who kindly examined the sections, stated that the hemorrhage was possibly agonal and in consequence may have borne no direct relation to the disease. This is suggested strongly by the fact that the urine of none of these patients contained blood, which should have been true had the hemorrhage existed for some time before death.

Unfortunately, there is no evidence available which indicates the degree of kidney damage necessary to cause retention. On the basis, however, of the lesions present in nephritis in adults with nonprotein nitrogen retention it would seem that in only two of our cases was the kidney lesion sufficient to account for the high nonprotein nitrogen. Lesions of similar grade to those found in the other cases are often

11. Hoffsten: *Virchow-Hirsch Jahresb.*, 1888, 11, 478.

12. Epstein: *Festschrift Edw. Hensch*, Berlin, 1890.

13. Bernhard and Felsenthal: *Arch. f. Kinderh.*, 1894, 17, 222.

14. Czerny and Moser: *Jahrb. f. Kinderh.*, 1894, 38, 430.

15. Jehle: *Jahrb. f. Kinderh.*, 1907, 65, 40, Part 1.

TABLE 8.  
*Evidences of Renal Involvement in Nine Cases of Intestinal Intoxication.*

Patient	Nonprotein Nitrogen, Mg. per 100 C.c. of Blood	Albumin in Urine	Casts in Urine	Evidence of Renal Lesions	
				Macroscopic	Microscopic
10*	61.9	+ Trace	Few hyaline many granular	Moderate congestion; no swelling of cortex; markings distinct	No significant lesions
13*	134.4	Heavy trace	Many hyaline granular and epithelial	Cortex pale, slightly swollen; markings slightly obscured	Many tubules contain red blood cells; tubular epithelium shows parenchymatous degeneration; apparent increase in nuclei in malpighian corpuscles, possibly due to shrinkage
14*	232.0	1 gm. per liter by Esbach	Many granular and epithelial†	Kidneys pale; greatly enlarged; edematous; cortex much swollen and markings obscured	Marked swelling and necrosis of tubular epithelium; foci of necrotic cells in tubules of cortex; coagulated material in glomeruli; apparent increase in number of cells in malpighian tufts; definite acute parenchymatous nephritis
6*	83.0	Trace	Many hyaline few granular	Kidneys slightly enlarged; cortex slightly swollen	Moderate swelling and parenchymatous degeneration of tubular epithelium; nuclei slightly pale
5*	150.0	Trace	Many hyaline few granular	Slight congestion	No significant lesions

P	113.4	Trace	Few hyaline many granular	Kidneys pale; slightly enlarged; cortex slightly swollen; slight edema	Many tubules contain red blood cells; parenchymatous degeneration of tubular epithelium.
H	65.8	Moderate 1.5 gm. per liter by Esbach	Many hyaline and granular	Kidneys pale; slight swelling of cortex	Marked hemorrhage into tubules of pyramids; hemorrhage into glomeruli; necrosis of tubular epithelium with the formation of a few necrotic foci in tubules; acute parenchymatous nephritis
V	90.3	Trace	Many hyaline few granular	Slight enlargement of kidneys; marked congestion	Hemorrhage into tubules; tubular epithelium shows moderate parenchymatous degeneration
O'D	68.0	Trace	Many hyaline few granular	Slight congestion	No significant lesions

\* These figures refer to the same patients as those in Tables 2 and 4.

† A few red blood cells in urine.



found in the absence of any chemical evidence of defective renal function.

It seems apparent, therefore, that the impairment of renal function in intestinal intoxication cannot be due in the majority of cases to a definite kidney lesion, but must depend on some other cause. It seems most probable that the cause lies in the depleted water supply of the body.

It is well established that water is the most efficient diuretic, and without an adequate supply secretion of urine is impossible. In intestinal intoxication the water supply of the body is usually depleted by the great loss of water in the stools. Many of the patients refuse to ingest even water and vomit water or food, so that there is but little fluid available to replace even the normal loss of water in the excretions. It seems probable, therefore, that the deficient elimination by the kidney is purely functional and is due to the fact that there is no water available for the formation of urine.

The fact that the increased concentration of the blood is insufficient to account for the high nonprotein nitrogen is in no way antagonistic to this idea. There are a number of factors which tend to keep normal the water composition of the blood.

Bang<sup>16</sup> found that when dogs are deprived of water the blood shows increased nonprotein nitrogen. The nonprotein nitrogen became normal when water was given in sufficient amounts. Bang gave no evidence bearing on the blood concentration. It seems quite possible that the high nonprotein nitrogen was due to defective elimination owing to lack of water.

The clinical importance of the great loss of water in intestinal intoxication is too well known to require special consideration. It is a matter of considerable difficulty to give these infants sufficient water to replace that lost in the excretions. In six cases of intoxication in which it was possible to give sufficient water approximately to replace that lost the excretion of urine was apparently normal and there was no significant increase in the nonprotein nitrogen of the blood. Of the same significance is the fact that in no case was there any material reduction in the nonprotein nitrogen of the blood before the normal excretion of urine was reestablished.

16. Bang, I.: *Biochem. Ztschr.*, 1915, **72**, 119.

From the evidence at our disposal it seems probable that the deficient kidney elimination in intestinal intoxication is dependent, to a great degree at least, on the negative water balance. Whether insufficient water in the blood and tissues is directly and entirely responsible for the diminished secretion of urine offers considerable field for discussion. There are a number of factors capable of affecting kidney function which may be of influence. It has been demonstrated by Starling<sup>17</sup> that the colloids of normal blood exert an osmotic pressure of 25 to 30 mm. of mercury and under these conditions the arterial pressure in the kidney glomeruli must be greater than 30 mm. of mercury to insure the formation of urine. A greater concentration of colloids would require a greater arterial pressure in the kidney for glomerular filtration to occur.

In intestinal intoxication it seems possible that the blood colloids may be increased to such degree that they exert an osmotic pressure greater than the pressure in the glomerular capillaries. If this were true the formation of urine would be prevented. Moreover, the total blood volume is probably reduced in cases of intestinal intoxication, which would diminish the blood flow through the kidney and consequently reduce the secretion of urine. Such conditions, however, would arise in great part, if not entirely, from the water deficit and at present have not been accurately determined.<sup>18</sup>

#### *V. The Acidosis of Intestinal Intoxication.*

Howland and Marriott<sup>19</sup> showed that acidosis is a frequent occurrence in severe diarrhea in infants. The frequency and degree of

17. Starling: Jour. Physiol., 1899, 24, 317.

18. There is no means of ascertaining the blood pressure in the kidney of human beings. In dogs it is assumed to be 20 per cent. less than the carotid blood pressure, but Cushny states that such assumption is little more than a guess (The Secretion of Urine, London, 1917). MacKenzie (Brit. Jour. Child. Dis., 1912, 9, 343) says that the anuria in severe diarrhea of infants is due to lowered blood pressure, although he gives no evidence to support this view. Hill (Arch. Pediat., 1913, 30, 588) has reported blood pressure determinations (femoral artery) in eighteen cases of severe diarrhea. His results show no reduction in blood pressure when compared with the values for normal infants obtained by Morse and Wyman (AM. JOUR. DIS. CHILD., 1914, 8, 270) and others.

19. Howland, J., and Marriott, W. McK.: AM. JOUR. DIS. CHILD., 1916, 11, 309.

acidosis in cases of intestinal intoxication has been discussed also by Schloss and Stetson.<sup>20</sup>

According to our present conception, acidosis is best defined as a reduction in the alkaline reserve of the blood. Our knowledge of the significance of the alkaline reserve and the factors which influence it is based on the work of Henderson<sup>21</sup> and Henderson and Palmer,<sup>22</sup> and has been discussed by Lundsgaard,<sup>23</sup> Peabody,<sup>24</sup> Hasselbalch and Gammeltoft,<sup>25</sup> Barcroft,<sup>26</sup> Howland and Marriott,<sup>27</sup> Van Slyke and Cullen<sup>28</sup> and others.

On the basis of this definition it is theoretically possible for acidosis to be due to several causes. In acidosis occurring with diarrhea Howland and Marriott<sup>19</sup> found no evidence that abnormal acids were formed. They found no significant increase in the acetone bodies in the blood, which demonstrated that the acidosis was not due to increased formation or diminished oxidation of these substances.

There is no evidence that the acidosis is caused by abnormal loss of alkali, for, as pointed out by Howland and Marriott, the analyses of diarrheal stools by Holt, Courtney and Fales show actually a loss of more acid than base. Nor can there be any significant loss of alkali in the urine, for both the hydrogen-ion concentration and the titratable acidity are increased.

Howland and Marriott concluded that the acidosis of diarrhea was probably dependent on defective elimination of acid by the kidney and suggested the possibility that it was due, in part, at least, to the failure of the kidney to eliminate acid sodium phosphate. Recently, they

20. Schloss, O. M., and Stetson, R. E.: *AM. JOUR. DIS. CHILD.*, 1917, 13, 218.

21. Henderson, L. J.: *Am. Jour. Physiol.*, 1908, 21, 427; *Ergebn. d. Physiol.*, 1909, 8, 254; *Jour. Biol. Chem.*, 1911, 9, 403.

22. Henderson, L. J., and Palmer, W. W.: *Jour. Biol. Chem.*, 1912-1913, 8, 393; 1913, 14, 81; 1914, 17, 305.

23. Lundsgaard, C.: *Biochem. Ztschr.*, 1912, 41, 149.

24. Peabody, F. W.: *Arch. Int. Med.*, 1914, 14, 236.

25. Hasselbalch, K. A., and Gammeltoft, S. A.: *Biochem. Ztschr.*, 1915, p. 205.

26. Barcroft, J.: *The Respiratory Function of the Blood*, Cambridge, 1914.

27. Howland, J., and Marriott, W. McK.: *Bull. Johns Hopkins Hosp.*, 1916, 27, 63.

28. Van Slyke, D. D., and Cullen, G. E.: *Jour. Biol. Chem.*, 1917, 30, 289.

have found that there is a considerable increase in the inorganic phosphorus in the blood, which is in accord with this view.<sup>29</sup>

The evidence derived from my own cases is entirely in accord with the view that the acidosis of intestinal intoxication is due to faulty elimination by the kidney. The blood has been examined for acetone bodies in six cases with severe acidosis, and in no case was the amount greater than could be accounted for by starvation. The greatest value was 37 mg. of total acetone bodies, determined as acetone, in 100 c.c. of blood.

In a great number of cases of relatively severe acidosis in intestinal intoxication, a single dose of sodium bicarbonate is sufficient permanently to correct the acidosis. It would seem most unlikely that this could occur if abnormal acids were being formed in large amounts. As an example the following protocol is given:

*Protocol 1.*—The patient, a boy, aged 6 months, was admitted Jan. 15, 1916. For two days before admission he had vomited almost every feeding and had many loose yellow stools. For twelve hours he had marked muscular twitchings, was semistuporous and refused all food. On admission the infant was somnolent, had distinct hyperpnea and seemed very ill.

Date	Carbon Dioxid Tension, Alveolar Air, Mm.	C.c. of CO <sub>2</sub> Reduced to 0.760 Mm., Bound as Bicarbonate by 100 C.c. of Blood Plasma (Van Slyke)
1/15	22	34.6
1/16	3 gm. sodium bicarbonate by intravenous injection	
1/16	40	80.0
1/18	39	72.0
1/20	41	69.0
1/25	37	68.0

The hyperpnea disappeared entirely after the bicarbonate and the stupor lessened but did not disappear. The diarrhea continued until January 20, and during this period the infant was toxic and very ill. Improvement began January 20 and January 25 the infant was practically free from symptoms. A single dose of alkali was therefore sufficient to eliminate entirely a marked degree of acidosis.

It might be suggested that in cases of this type the acid formation had perhaps occurred at a time before the patient came under observation and had ceased, so that the single dose of alkali was sufficient, merely because no acid formation was in progress at that time. Such

29. Personal communication, Dr. John Howland, Dec. 11, 1917.

objection, however, would be invalid in the cases in which the acidosis developed under observation. Five cases of this type have been studied, an example of which is given in illustration:

*Protocol 2.*—Case 22, boy, aged 10 months. The baby had from eight to ten loose stools daily for five days; the day before admission he refused all food and became somnolent. On admission he showed evidence of moderate fluid loss and his expression was dull. There was no hyperpnea. The development of acidosis is shown by the following data. On November 19 hyperpnea was noted.

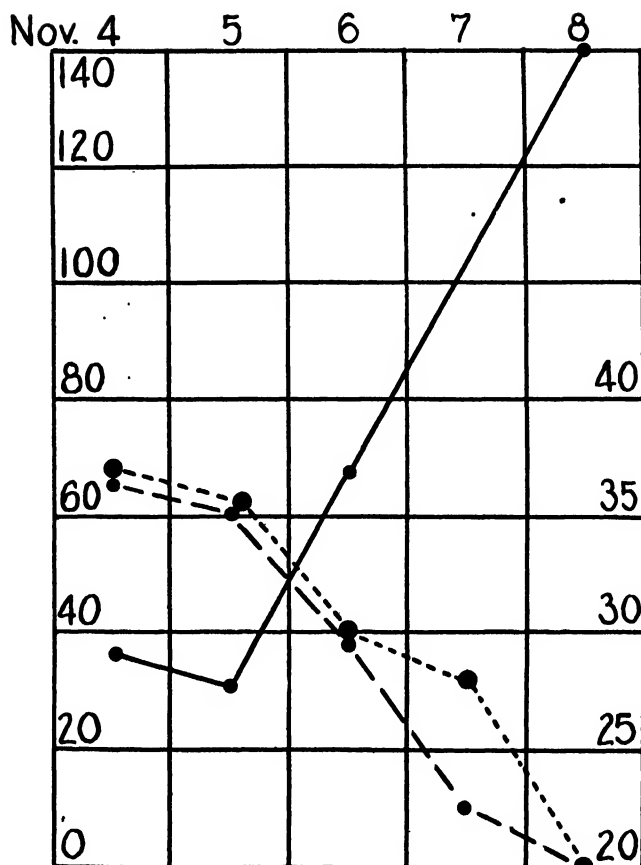
Date	Carbon Dioxid Tension, Alveolar Air, Mm.	C.c. of CO <sub>2</sub> , Reduced to 0.760 Mm., Bound as Bicarbonate by 100 C.c. of Blood Plasma (Van Slyke)
11/14/16	36.0	64.0
11/16	36.0	65.1
11/17	30.0	....
11/18	27.0	....
11/19	23.0	38.0
	3.7 gm. sodium bicarbonate by intravenous injection	
11/20	40.0	87.0
11/21	42.0	....
11/23	37.5	....
11/25	37.5	80.0
Death		

In this case the acidosis developed under observation and was corrected by the administration of sodium bicarbonate. The hyperpnea disappeared; the other evidences of intoxication were influenced but little, and the infant died without further evidence of acidosis.

These observations are entirely in accord with the view that the acidosis is due to the failure of the kidney properly to fulfil its function of preserving the alkali reserve of the blood and contrary to its dependence on the formation of abnormal acids.

Evidence has been given which demonstrates that there is an accumulation of waste products in the blood due to defective secretion of urine. It seems only reasonable to suppose that the acidosis is of similar origin.

Six patients with intestinal intoxication have been observed to whom it was possible to supply approximately as much water as was lost. None of these patients showed a marked diminution in the excretion of urine and none developed acidosis.



Baby Y., aged 9 months. For three days previous to admission he appeared ill and passed from three to four loose green stools a day. During this period he vomited four times. On admission he was fairly nourished, the fontanel was not depressed and the skin was fairly moist and elastic. The infant was somnolent but could be roused easily. Until November 7 the food consisted of 3 ounces each of protein milk and barley gruel, given every four hours. In addition, large amounts of water were given. The stools were watery and from five to seven were passed in twenty-four hours. Vomiting became severe on November 8 and the food was changed to barley gruel. In the chart ----- with the figures at the left = urine in c.c. during a two-hour period after ingestion of 120 c.c. of water; ————— = nonprotein nitrogen of the blood, in milligrams per 100 c.c.; ..... with the figures at the left = carbon dioxide tension, in millimeters, of the alveolar air.

In five cases the acidosis developed under observation. In three the output of urine was carefully observed, and in each instance the acidosis was preceded by a diminished excretion of urine and the chemical evidence of impaired renal function. This occurrence is illustrated by the accompanying curve.

It would seem, therefore, that the acidosis of intestinal intoxication is probably due to defective kidney function.

#### *VI. The Symptoms of Intestinal Intoxication.*

To determine definitely at present the origin of the symptoms of intestinal intoxication is obviously impossible, but there are facts bearing on this question which seem of sufficient importance to mention.

In a number of cases the acidosis bears a definite relation to the symptoms. This is quite evident when the symptoms disappear promptly after the correction of the acidosis by alkali. In some of the cases there is but little improvement in the symptoms after acidosis is eliminated. Hyperpnea, if present, always disappears, but at times the patient remains semistuporous and the prostration and other manifestations remain unchanged. Although in such cases the symptoms cannot be due directly to acidosis, yet it seems possible that the lack of alkaline reserve had caused injury to the tissue which is not repaired by the mere correction of the existing acidosis. Even granting the correctness of this assumption, there are cases of intestinal intoxication in which the symptoms are marked and yet acidosis is absent. The symptoms in these cases, except that hyperpnea is absent, seem identical with those of cases in which acidosis is present. This is also true of those cases in which the symptoms improve after the administration of alkali but return within a short time despite the fact that there is no recurrence of acidosis.

The improvement which occurs after the administration of sodium bicarbonate is probably not always due entirely to the alkali. Part is due to the water, and moreover, in the regular treatment of these cases, large amounts of water are given independently of alkali.

While there seems no question that acidosis is an important cause of the symptoms of intestinal intoxication, yet it is by no means the whole cause.

There is no evidence to indicate that the retained nitrogenous waste products are responsible for the symptoms. It is generally believed

that in uremia, even with considerable nonprotein nitrogen retention, these substances are not responsible to the toxic symptoms. Recently, Hewlett, Gilbert and Wickett<sup>30</sup> were able to cause toxic symptoms in normal adults by feeding large amounts of urea. The symptoms, however, were slight in comparison to the degree of urea retention in the blood and as compared with what is often observed in uremia. These experiments show that large amounts of retained urea can be toxic, yet they do not demonstrate that the amount of urea retained in intestinal intoxication can be the cause of the toxemia.

There is a close analogy between intestinal intoxication and uremia. Indeed, all of the evidence points directly to the fact that their nature is essentially the same. In uremia the kidney fails to fulfil its proper function, due to an anatomic lesion. In intestinal intoxication the same result is brought about through loss of fluid. Waste products normally excreted by the kidneys are retained because there is insufficient water for diuresis.

Similar to uremia, based on symptomatology, there are two groups of cases of intestinal intoxication. In one group convulsions, involuntary muscular movements, muscular twitching and coma are present. The onset is usually acute and the infant is not greatly emaciated. Diarrhea may be slight and is absent in some cases. Food or water is usually refused and is vomited if taken.

In the second group the onset is less acute, but occurs following vomiting and diarrhea of some days' duration. Convulsions rarely occur and the infant is somnolent or semistuporous instead of comatose. Wasting is very marked and the patient presents the shrunken appearance which is so characteristic of great loss of fluid.

In both uremia and intestinal intoxication acidosis is a frequent occurrence.

Aside from the question of acidosis, intestinal intoxication similar to uremia appears to be some form of poisoning. Whether this poison is a product which the kidney has failed to excrete, whether it arises from disordered metabolism or is elaborated by bacterial growth in the intestinal tract, is one of the fundamental problems with which we are confronted.

30. Hewlett, A. W., Gilbert, Q. O., and Wickett, A. D.: *Arch. Int. Med.*, 1916, 18, 636.



## SUMMARY.

In intestinal intoxication there is a marked increase in the non-protein nitrogen and urea of the blood. This increase is not due directly to increased concentration of the blood from loss of water. The high nonprotein nitrogen and urea are due to defective kidney elimination.

The renal lesions in intestinal intoxication are not sufficient to account for the impaired elimination by the kidney. It is probably due to the fact that lack of water restricts the formation of urine. This condition is probably due to the following factors which may act singly or in combination: 1. The loss of water in the stools is so great that it is impossible for the infant to ingest sufficient fluid to replace the loss. 2. The patient refuses to ingest fluid or vomits practically all that is taken. As a result the tissues become dehydrated. The retention of nitrogenous waste products and the failure of the kidney to do its part in preserving the acid-base equilibrium results from the deficient secretion of urine. The oliguria may be due to the fact that the dehydrated tissues hold as much water as possible so that none is available for the formation of urine. Other factors, dependent mainly on the loss of water, which may play a rôle are: 1, an increase in the concentration of blood colloids to such degree that their osmotic pressure is greater than the arterial pressure in the kidney; 2, a diminution in the total blood volume leading to decreased blood flow through the kidney.

The symptoms of intestinal intoxication are essentially those of uremia. The two conditions are similar in all essentials. In one, defective kidney function is due to an organic lesion; in the other it results from the negative water balance and consequent oliguria.

Acidosis plays a definite part in the symptomatology of intestinal intoxication, but the essential cause is probably some unknown toxic agency.

It is with pleasure that I acknowledge my great indebtedness to Dr. L. E. La Féra for the use of material from his service and for his stimulating interest in the work. I desire to thank Dr. Charles Norris for examining and describing the kidney sections. To Dr. A. O. Gettler, pathologic chemist, Bellevue Hospital, I am indebted for much advice and assistance. My thanks are due to Dr. W. R. May for much assistance during the early part of the work.

## A STUDY OF THE BLOOD PRESSURE BY THE METHOD OF GAERTNER, ESPECIALLY IN PATIENTS SUFFERING FROM FIBRILLATION OF THE AURICLES.

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### INTRODUCTION.

A number of years ago Mackenzie (1) called attention to the difficulty in estimating the blood pressure in persons suffering from fibrillation of the auricles. There was general agreement that the difficulty existed, but there seemed to be no method for improving the technique of making clinical determinations. The beats which were heard first by auscultation or felt first on palpation, when pressure in the cuff system was allowed to escape, continued to be taken as the measure of the systolic pressure. A method was then suggested by James and Hart (2), later called the fractional method by Kilgore (3), by which not the maximum pressure of a few beats is taken as the measure of pressure, but the average of the systolic pressures of all the beats. Their technique consists in counting for 1 minute the number of beats felt below the brachial cuff at the radial artery at levels 10 mm. of mercury apart. The figure obtained is multiplied by the level of pressure, all the products are then added, and the sum is divided by the apex rate. Slight modifications and certain extensions of the method have since been suggested by Kilgore. He employed auscultation in addition to palpation, especially in calculating diastolic pressure.

This method presents certain difficulties, but before considering them we wish to review again certain points underlying the current clinical technique of estimating blood pressure. The peripheral arteries are regarded as representing an elastic reservoir kept filled by the action of the heart. It is the tendency of this reservoir to return to a position of rest, and in doing so to empty itself continuously into the smallest arteries and capillaries. By this effort these vessels are kept constantly filled.<sup>1</sup> So far as the constancy of the filling is concerned it makes no difference whether the elastic reservoir is filled by

<sup>1</sup> Cases in which there is a capillary pulse are exceptions to this rule.

volumes of blood equal in amount as normally, or unequal as in fibrillation of the auricles; or whether these volumes are delivered at equal or at somewhat unequal intervals. It matters only that the total volume in succeeding units of time, minutes for instance, is kept constant. Under ordinary circumstances this maintenance of constant volume is accomplished by uniform filling, but, failing this, it can be brought about by a compensating increase (or decrease) of resistance offered at the outflow of the reservoir system. It is an advantage to have the minute volume output of the left ventricle divided in roughly equal fractions throughout the minute. The point at which the reservoir empties into the smaller vessels may be regarded as the point where the head of pressure in the reservoir becomes effective. We speak then of the "effective pressure"<sup>2</sup> at this point.

The clinical habit of estimating arterial pressure in the brachial artery is adopted, not because the pressure here is especially important, but because it serves, on the one hand, as a rough indicator for the pressure level at the root of the aorta or in the left ventricle itself; that is, a difference is assumed between aortic and brachial pressure, sufficiently constant to permit the brachial pressure to serve as a valuable guide. On the other hand, brachial pressure may also serve as a suitable guide for judging the level of effective pressure, the pressure at the exit from the arterial into the capillary system. So far as the capillary system is concerned, there is no difficulty in this convention as long as the filling of the reservoir is maintained by even and uniform strokes; when the strokes are no longer uniform as in fibrillation, the difference between effective pressure, which tends to stay constant or alters only within narrow limits, and brachial pressure, which varies, must fluctuate so that the brachial pressure can no longer serve as an index of effective pressure.<sup>3</sup>

These considerations are important in relation to the technique of estimating systolic pressure when the auricles are fibrillating, for it is precisely in this condition that brachial pressure fluctuates and fails as an index of effective pressure. It has been found on closer study (3) that in auricular fibrillation not only the systolic, but also

<sup>2</sup> This phrase is used by James and Hart (2).

<sup>3</sup> See experiments reported by Cohn, A. E., and Lundsgaard, C., *J. Exp. Med.*, 1918, xxvii, 505.

the diastolic pressure of succeeding beats varies. These fluctuations necessarily result in irregularities in the pulse pressure. The pulse pressure does, in fact, undergo large alterations in the brachial artery. Further toward the periphery, however, the pulse pressure becomes progressively smaller, until, in the capillaries, it ceases to exist<sup>1</sup> and the flow is constant and continuous. The point at which this takes place is the point where, as has been said, the head of pressure becomes effective. Here a constant reading can be obtained. But since for clinical purposes no practicable technique exists for this, a point just proximal to the capillaries may be chosen. In the small arteries, of the size of the digital, the pulse pressure is small and alterations from mean pressure can from beat to beat be shown to be relatively unimportant.<sup>4</sup> For taking the pressure at this point a technique already exists. It is the method formerly employed by Gaertner and described by him in 1899 (4). The values obtained may be regarded as effective blood pressure. Readings so obtained are direct and the technique is simple. By this method we have taken the blood pressure of a few persons suffering from fibrillation of the auricles and have plotted, parallel to these curves, others made day after day by the fractional method of James and Hart, with the view of ascertaining the difference between the brachial and digital pressures. We have, in addition, studied a number of other individuals, some with normal and some with abnormal hearts, but all having hearts the mechanism of which was normal.

#### *Technique.*

The following technique was employed. One finger,—the ring finger,—was rendered bloodless by rolling a thick rubber ring from the tip to a small pneumatic cuff which was applied to the first (base) phalanx. The cuff corresponded exactly in plan to that of von Recklinghausen except that it was 2.5 cm. wide and about 5 cm. long. The cuff was connected by pressure tubing to a mercury manometer as in the von Recklinghausen plan. The pressure in this system was raised with a pump after the finger was blanched by the rubber ring. The ring was then removed and the pressure in the manometer cuff system

<sup>4</sup>This point is described in our report, *J. Exp. Med.*, 1918, xxvii, 505.

allowed to fall gradually and regularly.<sup>5</sup> When the pressure in this system falls to the level of the blood pressure, blood begins to flow into the finger distal to the cuff and is recognized by the return of color in the finger. After a little practice there is no difficulty in recognizing the return of color. It is important to make the estimations with north light. We have been aided in seeing the return of color, even in the very anemic, by laying the finger and hand to be examined on a dark, blue-gray cloth, to provide a proper contrast. It was the custom to read the pressure in each case 10 times and to average the readings. Of 153 determinations the range was 1 to 5 mm. in 11 determinations; 6 to 8 mm. in 69; 9 to 10 mm. in 35; 11 to 15 mm. in 27; 16 to 30 mm. in 7; 21 to 25 mm. in 2; and 26 to 30 mm. in 2. That is, the range was below 10 mm. in 75 per cent of the determinations. In many instances both observers made independent readings. When that was done, we compared the averages in twenty-six instances and found a difference of 2 to 4 mm. Twice the averages differed by 5 mm.; twice by 6 mm.; once by 7 mm.; twice by 8 mm.; once by 9 mm.; and once by 11 mm. The differences in range are greater than were found in the estimation of brachial pressure by Kilgore and his associates (5) but not so great as to render the method unserviceable. As an example of the method followed, we cite the figures of one patient, Case 2334 (Tables I and II), made by two observers.

Studies of blood pressure by Gaertner's method have been made with the view of comparing the readings found at the digital, with those taken at the brachial artery.

Hayashi (6), in Strümpell's clinic, found a difference of about 20 mm. between the readings taken of these two arteries whether they were made in children, men, or women, in infections, or in cardiac, vascular, or renal diseases. There were fluctuations in his figures but these were not of sufficient importance to alter the conclusion that the fall in pressure from the brachial to the digital vessels was about 20 mm. Doleschal (7) compared the pressure in the radial artery

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<sup>5</sup> We provided for the gradual and regular fall in pressure by placing the rubber bulb which served as a pump between the jaws of a wooden vice. The distance between the jaws was regulated by a screw turned by a long shank; the use of a long shank permits more uniform motion. As the screw was released, the jaws of the vice were separated by springs properly placed.

TABLE I.

March 26, 1917.

Tonometer readings.	
No. 1.	No. 2.
<i>mm. Hg</i>	<i>mm. Hg</i>
93	93
89	95
92	95
88	95
87	93
91	93
94	92
95	93
94	95
93	92
Average.....91.6 = $92^{+3}_{-4}$	93.6 = $94^{+1}_{-2}$

TABLE II.

Blood pressure by the fractional method.			
Auscultation.		Palpation.	
Pressure.	No. of beats heard.	No. of beats felt.	Pressure.
<i>mm. Hg</i>			<i>mm. Hg</i>
140	0	0	
130	31	20	$130 \times 20 = 2,600$
120	25	22	$120 \times 2 = 240$
110	9	26	$110 \times 4 = 440$
100	9	61	$100 \times 35 = 3,500$
			<u>6,780</u>
90	21	59	
80	18	61	
70	7	58	
60	2	60	$\frac{6780}{62} = 109$
50	0	56	
Average pressure.....130		109	
Radial rate..... 62			
Apical " ..... 62			

taken by means of von Basch's sphygmomanometer with that taken by Gaertner's tonometer and found uniformly that the tonometer readings were lower than those taken with von Basch's instrument. These results were naturally to be expected, and show that even where the pressure differences are probably small, as in the case of the radial and digital arteries, the tonometer readings, as we anticipate, are lower. Of 200 cases, for instance, the readings showed differences up to 5 mm. in 89, to 10 mm. in 80, to 15 mm. in 19, to 20 mm. in 6, and from 20 to 40 mm. in 6.

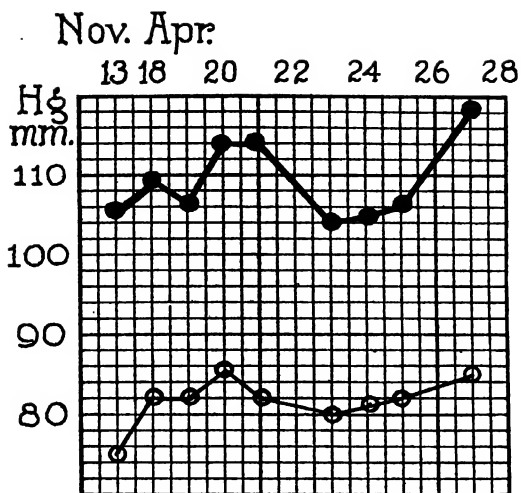
Our experience corresponds closely with that of Hayashi and Dolechal, for we have found uniformly that the tonometer readings at the digital arteries are always lower than the brachial readings. In eight normal individuals we found the average difference to be 20 (19.8) mm., the maximum being 30 mm., the minimum 5 mm. (Table III).

TABLE III.

No. of individual.	Brachial systolic pressure by auscultation.	Tonometer.	Difference.
	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	110	96	14
2	120	92	28
3	116	97	19
4	119	98	21
5	110	96	14
6	114	109	5
7	120	90	30
8	109	81	28
Average.....			19.8

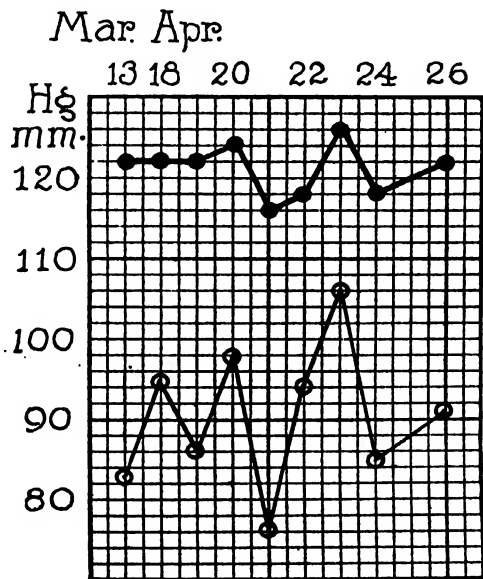
In the patients suffering from chronic heart disease and chronic nephritis (Table IV), in whom the mechanism of the heart beat was normal, the average difference between the two was 25 mm. in all cases, the range being from 6 to 80. If two cases (Nos. 10 and 12), suffering from complete heart block, are omitted, the average is 18 and the range 6 to 30. These values do not differ from normal.

If the cases are arranged according to the height of the brachial pressure, no correspondence between this and digital pressure is observed. The reason probably depends on the instability known to exist in the vasomotor mechanism in different individuals. It is pre-



- Brachial pressure estimated by the method of auscultation.  
 - - - Brachial pressure estimated by palpation.  
 — Digital pressure.

TEXT-FIG. 1. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.



TEXT-FIG. 2. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.



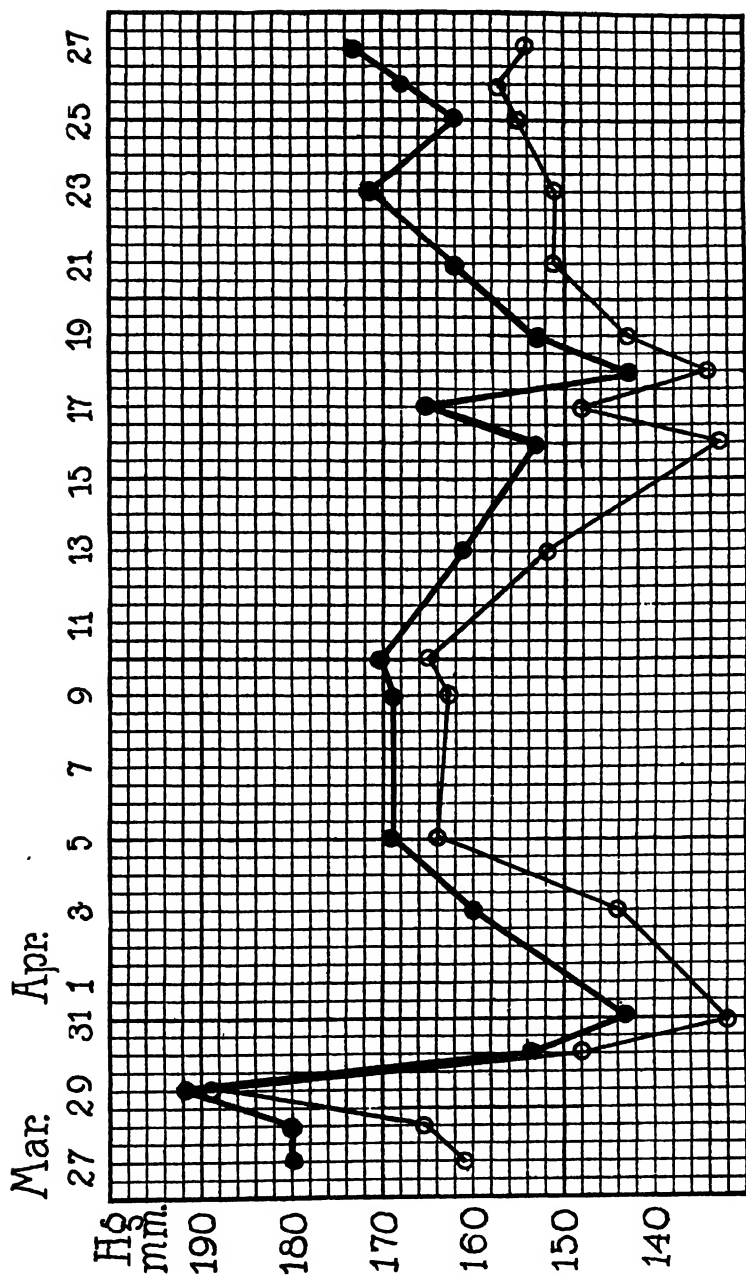
cisely in the smaller vessels that lability is observed, so that fluctuation is expected in the digital rather than in the brachial artery. It is for this reason that the readings in the two arteries are not parallel. In some respects the violent fluctuations in digital pressure present difficulties, so that as a guide for comparison pressure in the digital artery is unsatisfactory. The question arises, however, whether the difficulty which is found is not of clinical importance, and whether it ought not to be emphasized rather than evaded entirely as is now done in estimating pressure only at a point such as the brachial ar-

TABLE IV.

Case No.	Hospital No.	Rate.	Brachial systolic pressure.	Tonometer.	Difference.
			<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	587	80	84	65	19
2	2915	86	100	84	16
3	2069	109	103	73	30
4	2681	96	145	126	19
5	2867	83	134	106	28
6	2862	82	145	128	17
7	2961	90	153	147	6
8	2907	82	160	139	21
9	2336	85	176	169	7
10	1266	30	176	135	41
11	2992	92	180	162	18
12	2833	30	250	170	80
Average.....					25.1

tery where the least variation is found. We recall that the method of Gaertner was practically abandoned because of the fluctuations inherent not so much in the technique as in the artery itself. Furthermore, the matter is important on account of questions associated with fluctuations in oxygen unsaturation in the venous blood of the arm such as Lundsgaard (8) has found. These phenomena may find their explanation, in part, in facts like those which are shown here.

Curves of the brachial and digital pressure of two individuals in both of whom the circulation was normal illustrate these points. In the first instance (Text-fig. 1) the pressure in the brachial artery fluctuated within the usual narrow limits from 104 to 118 mm., a range of



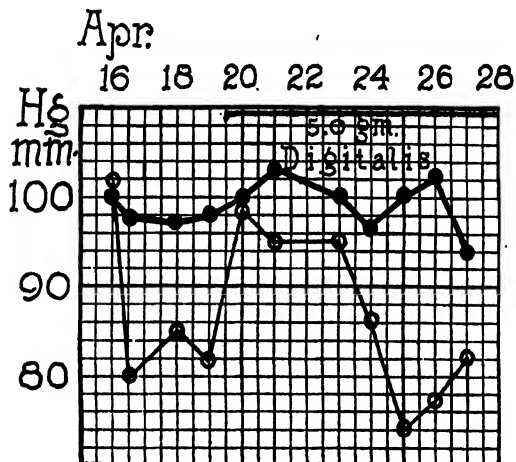
TEXT-FIG. 3. Curves of the brachial and digital pressure of a patient suffering from chronic nephritis and heart failure.

14 mm. Parallel digital pressure fluctuated between 75 and 85 mm., a range of 10 mm. The digital pressure was more constant than the brachial. In the second instance (Text-fig. 2) the brachial pressure fluctuated between 116 and 126 mm., a range of 10 mm., while the digital pressure fluctuated between 76 and 106 mm., a range of 30 mm., or three times as great as the brachial pressure. We expected the peripheral circulation of the first individual to be stable, but in the second we anticipated, because of the frequent rapid alterations of vasomotor tone seen in phenomena like dermatographia, that it was labile. Studies of blood flow, or oxygen unsaturation, already mentioned, had indicated that phenomena of the nature we are now emphasizing, existed to explain the obscure facts relating to flow. There is reason, therefore, to think that in studies like this, facts of importance may be found.

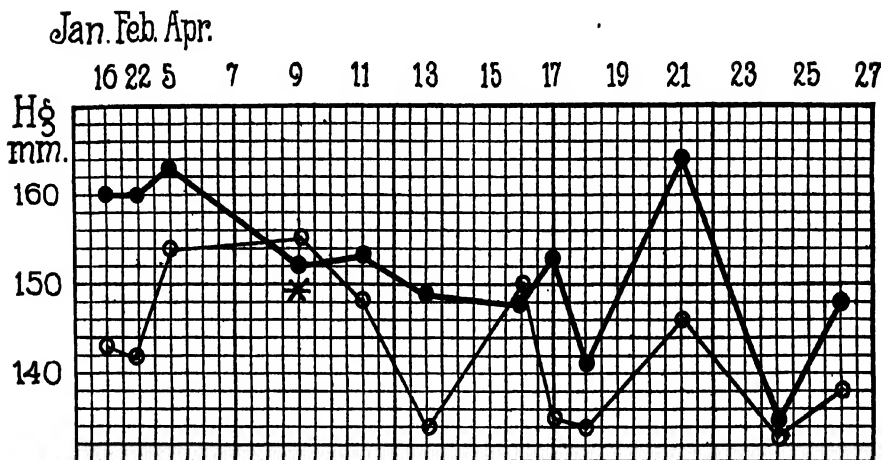
In this connection a consideration of the records of a patient (Text-fig. 3) are important. This individual suffered from chronic nephritis and heart failure; he was at first edematous and orthopneic and had a high pressure and an alternating pulse. The digital pressure fluctuated between 132 and 189 mm., a range of 57 mm. But the brachial systolic pressure fluctuated almost as violently between 143 and 192 mm., a range of 49 mm. In this instance the fluctuations are almost parallel and indicate that they depend on alterations in the functional state of the heart rather than on fluctuations in vasomotor tone. These three cases show that there are instances in which simultaneous brachial and digital readings are of value. But we have cited them in addition to show that of the two arteries, the blood pressure is uniformly higher in the one more centrally placed; the curves of the two do not cross.

#### OBSERVATIONS.

Our especial interest was directed to the study of the relation of digital to brachial pressures in individuals suffering from fibrillation of the auricles. We studied four patients in detail. The pressure curves made by the tonometer method are not remarkably different from those found when the mechanism of the heart beat is normal. But when the digital pressure curves are compared with the brachial pressures obtained by the method of fractional readings devised by



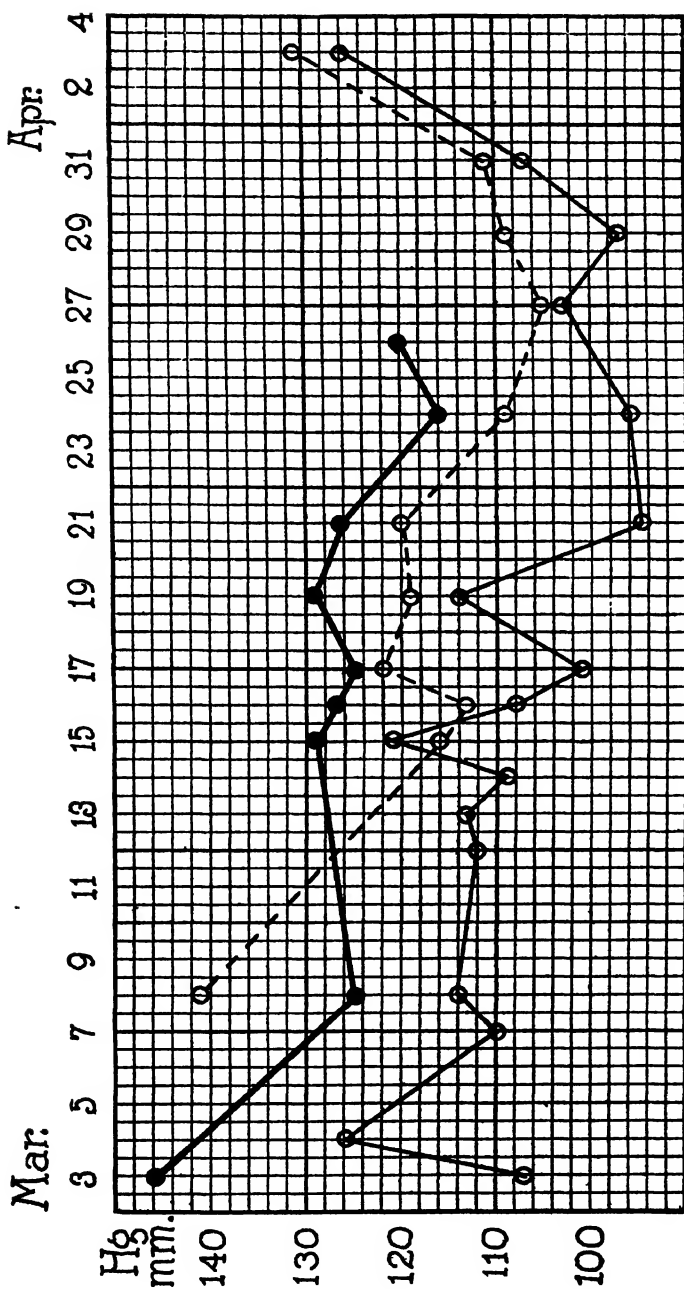
TEXT-FIG. 4. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once.



TEXT-FIG. 5. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross twice. The upper curve represents pressure in the brachial artery determined by the method of auscultation, except where indicated by an asterisk. The lower curve represents digital pressure taken by Gaertner's method.

James and Hart, difficulties occur. In the first place, the taking of readings requires as many minutes or half minutes as there are levels at which pressure is read. During this time the state of the vessels does not remain the same, the number of sounds heard or beats felt at one level may, after 3, 4, or 5 minutes, alter appreciably; sometimes, indeed, no reliable count is possible. To avoid continuous pressure, we invariably reduced the pressure to zero, for at least 1 minute between successive readings, although the length of the examination was thereby doubled. The procedure, however, did not remove the difficulty. The explanation for this may depend on a number of causes related to the auscultatory method, possibly to the formation of the sounds. But another cause also deserves consideration. Compression of the brachial vessels for so long a time may alter the partition of the blood stream so that an increasing fraction passes, uncompressed by the cuff, in the medullary vessels of the bone or in vessels protected by the grooves on its surface. This explanation implies a rapid increase in the area of the collateral vessels, but there is reason for believing that it may take place. It has, on the other hand, been observed that the difficulty may develop gradually. If absent at first, it increases as the daily examinations proceed. The establishment of the collateral circulation need, therefore, not be abrupt. The factors involved in the production of the sounds, whether produced by the mechanism of the water-hammer or otherwise, may also change. Whether these are the occurrences which actually take place and are the ones responsible for the difficulty we are considering, is of secondary importance. The sounds in any even do not remain sufficiently distinct in many individuals to make auscultation reliable as the basis of the fractional method. For the reasons stated, counting the beats by palpating the radial artery also offers difficulties. We have, therefore, in two cases counted the rate in the radial artery by palpation as well as in the brachial by auscultation. In one case in which it was satisfactory, we used the auscultatory method alone.

A second difficulty is that the curve of average brachial pressure occasionally crosses and is lower than the level of digital pressure taken on the same day. A crossing of the two curves had not been observed by others and was not seen in our studies of individuals the



TEXT-FIG. 6. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once when the fractional pressure by palpation is considered.

mechanism of whose hearts was normal. The crossing of the curves is, moreover, inconceivable. In Case 13 (Table V) crossing was observed once in eleven observations (Text-fig. 4); in Case 14 (Text-fig. 5) twice in twelve observations; in Case 15 (Text-fig. 6) once in seventeen observations when the fractional pressure was taken by palpation, but not if taken by auscultation; in Case 16 (Text-fig. 7) thirteen times (in two they were equal) in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation.

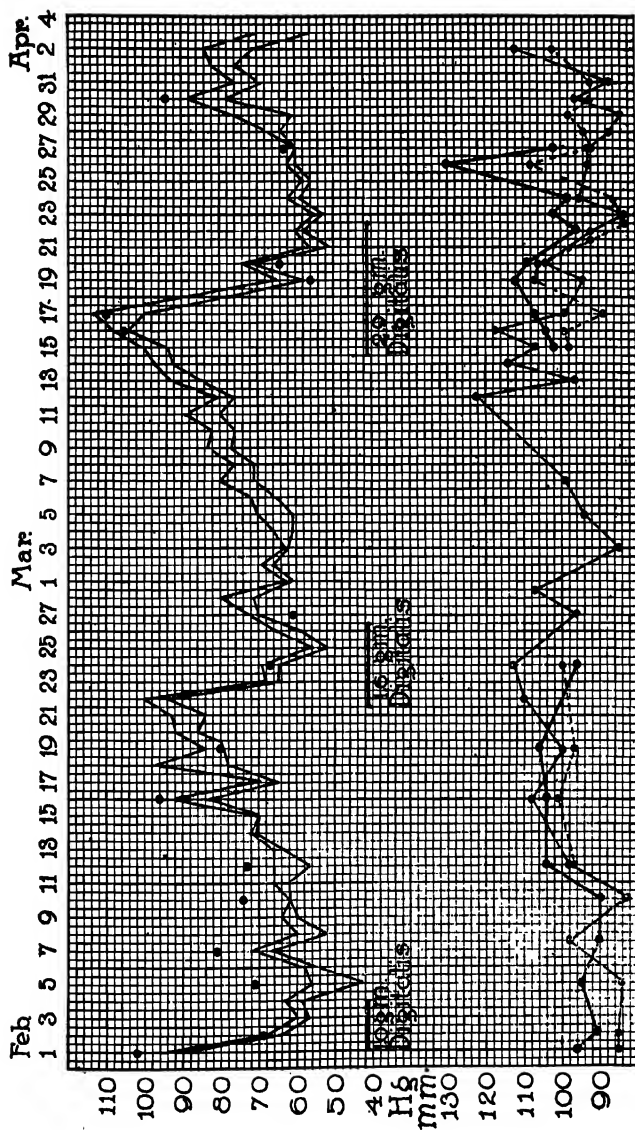
TABLE V.

Case No.	Hospital No.	No. of observations.	Crossing.	
			Observed by method of palpation.	Observed by method of auscultation.
13	3007	11		1
14	2548	12	1	1
15	2683	17	1	
16	2334	{ 19 20	13	5

Although the number of patients studied is not large, the fact that in each of them the fractional method of estimating pressure gave readings for the brachial artery lower than for the digital is important. The finding makes it desirable to review the opportunities for error.

An average pressure can no doubt be obtained by the fractional method. But even if the result is obtained free from the technical errors already discussed, and from one other now to be considered, it would probably be incorrect because it is not possible to regard the circulation as is usual in physical phenomena. In order to do this the value of one cycle ought to be directly comparable with another in terms of its numerical equivalent.<sup>6</sup> To regard it in this manner is, however, impossible. If, for instance, a ventricular cycle is unable to lift the aortic valves, this cycle is of no importance in increasing or even in maintaining the pressure in the arterial reservoir. It has no functional value and ought not to be included in calculating the average pressure; it tends merely to lower the value. The only

<sup>6</sup> Kilgore has considered this matter in a similar manner.



TEXT-FIG. 7. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross thirteen times in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation. In the upper set of curves the upper line represents the average ventricular rate for the day, the lower one, the average radial rate. The dots indicate the ventricular rate at the time of blood pressure determination. In the lower set of curves the brachial pressure by the auscultatory method is represented by the heavy line; brachial pressure by the method of palpation is represented by the broken line. Digital pressure taken by the method of Gaertner is represented by the light line.



ventricular cycles which can have an influence on the pressure in the arteries are those the systolic value of which is greater than diastolic pressure. But as far as maintaining the driving force of the circulation is concerned, that is the head of pressure represented by the difference between systolic and diastolic pressure, not all the cycles having a systolic value above diastolic pressure are equal. Many having a low value need have no significance in maintaining the effective pressure. In cases where the rate is low, and where there is no pulse deficit, whether the rhythm is regular or irregular, both a high systolic and a high diastolic level can be maintained. This is observed in cases of complete heart block. Of two such cases studied, the brachial systolic pressure in one was 250, the diastolic 90, and the digital pressure 170; and in the other the systolic pressure was 176, the diastolic 63, and the digital pressure 164. It is apparent that additional ventricular beats, if they are ineffective, whether able to lift the aortic valves or not, would have no significant influence on these figures. The rate in both cases was about 30. A few cycles of great power can therefore maintain a high head of pressure. We infer from this that 30 to 40 beats at a given pressure, 130 mm. for instance, are sufficient to maintain effective pressure close to this level. If additional 40 to 60 beats having low systolic pressure values are introduced into the calculation the numerical value given to the pressure is merely reduced and no added light is thrown on the actual circulatory condition. And if ventricular cycles that do not even develop force sufficient to open the aortic valves are included, the average pressure is still further depressed.

In estimating the systolic pressure in fibrillation of the auricles, therefore, the fractional method is defective. A substitute is readily found in the use of the tonometer method of Gaertner. By its means satisfactory readings are possible. One should remember, however, that the readings so obtained tend to be about 20 mm. lower than the pressure in the brachial artery. As a rough method for estimating the pressure in fibrillation, it appears to us to be sufficient to find rapidly the level of pressure in the brachial artery at which about 40 beats per minute occur; this point is not far removed from the effective pressure about which information is actually required. It avoids, furthermore, the appearance of numerical accuracy, where accuracy, as Mackenzie pointed out, is impossible.

In two patients (Nos. 13 and 16), the subjects of fibrillation of the auricles, we were able to study the effect of digitalis on the brachial and digital pressures. In the first, after giving digitalis for about 8 days, there was a slight lowering of the digital curve (Text-fig. 4) from 97 to 74 mm. But in the period before this, readings of 80 to 85 mm. were recorded and a reading of 84 was found later in the treatment. The influence of digitalis in this instance cannot, therefore, be called striking. In the second case (Text-fig. 7) there were three digitalis periods separated by 14 or more days during which 1, 1.6, and 2.9 gm. were given. In each digitalis period the digital pressure fell, rising again during the intervals between administrations. In the first interval the pressure rose from 83 to 108 mm.; in the second interval from 85 to 122 mm.; in the third interval from 83 to 112 mm. It is the usual experience, when the mechanism of the heart is normal, to find little or no alteration in brachial blood pressure during digitalis administration. Whether this is true when the auricles are fibrillating, is unknown. The fractional blood pressure curve in this patient taken either by palpation or auscultation is unsatisfactory, so that the record of its behavior is not valuable. Nor is it possible to construct a brachial curve based on the levels at which about 40 beats were heard or felt because the counts due to the long examination made necessary by the fractional method are inaccurate. These observations permit us to state merely that we observed not a rise but actually a fall in digital pressure during digitalis administration. The subject requires further study.

#### SUMMARY.

The function of the arteries as an elastic reservoir between the heart and the capillaries is reviewed. The appropriateness of selecting the exit from this reservoir as the point for estimating its effective pressure is shown. The technique for taking the pressure here by the method of Gaertner is described and its advantage and certain apparent disadvantages are indicated.

The technique of Gaertner is shown to be especially applicable to the study of the blood pressure in fibrillation of the auricles. The use of this technique has brought out a defect in the so called fractional method of taking the pressure in this condition; the brachial and digital curves cross.

Taking pressure of both brachial and digital arteries has shown that certain different types exist; first, that in which both central and peripheral pressures are stable; second, that in which the more central pressure is stable and the peripheral pressure fluctuates; and third, that in which both pressures fluctuate together.

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## THE PERIPHERAL BLOOD PRESSURE IN FIBRILLATION OF THE AURICLES.

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### PLATE 11.

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In a study of the blood pressure in persons whose auricles were fibrillating we found that satisfactory readings can be obtained at the digital artery by the method of Gaertner (1). It is well-known that the pressure in the brachial artery in auricular fibrillation is difficult to take either by the auscultatory or palpatory method on account of the large differences in pulse pressure of succeeding beats. The method of taking the pressure at the digital artery is more likely to be satisfactory, because the pulse pressure is much smaller at this point and the fluctuations from beat to beat, compared with those in the brachial artery, are probably insignificant.

### EXPERIMENTAL.

To determine this point we performed the experiments reported below. Dogs were anesthetized with morphine, paraldehyde, and ether. The right femoral artery was connected with a membrane manometer; the small artery on the dorsum of the left hind foot, with a mercury manometer. Two serrefines, to each of which insulated copper wire was soldered, were fastened to the right auricular appendix. They were introduced into the chest through a small intercostal incision. The other ends of the wires were attached to the secondary coil of an inductorium. The auricles were made to fibrillate by stimulating them with an interrupted current, and the action of the ventricles thereupon became completely irregular.

The curves which we obtained show that when the auricles were in a state of fibrillation, the rate of the ventricles rose, in this case from 123 before the inception of this rhythm to 210 (Fig. 1). The

pulse pressure in the right femoral artery fluctuated through a wide range. But in the artery of the dorsum of the left hind foot the fluctuations in pulse pressure were small and did not vary by more than a few millimeters of mercury. It is clear, therefore, that the pressure here does not vary through so wide a range as in the larger vessels; the value of any one beat differs but slightly from that of the others. In view of the small pulse pressure, the systolic and diastolic limits are not far removed from the mean pressure. The readings are simply and directly made. The experiments show, therefore, that direct readings, not far removed from mean pressure, may be obtained in dogs from the small arteries. In man the pressure conditions in the small arteries, like the digital, are doubtless similar to those in the dog. With a technique such as that recommended by Gaertner, satisfactory estimates of pressure should accordingly be possible. Such estimates have been made and the results obtained are published elsewhere (1).

Our experiments bring out, in addition, certain points bearing on the blood pressure when the auricles fibrillate. In the curve reproduced, the fact appears that the general level of the pressure in the smaller vessel does not fall, but on the contrary tends, on the whole, to rise slightly. A similar rise, probably temporary, was observed in three of the four experiments; the pressure fell in only one. In two of the three instances in which the pressure rose at first, it fell later in the experiment, after prolonged anesthesia and operative manipulation. The behavior of the pressure during experimental auricular fibrillation is of interest to us as it was to Lewis (2), because of the bearing of the experimental data on the phenomena in man. Lewis observed that "the disturbances of the circulation are so profound in the human subject, when this curious disorder of the heart's action begins" that he was impelled on this account to investigate the nature of the new conditions experimentally. We too have seen profound disturbances in the circulation under these conditions, but there has, on the other hand, been abundant opportunity to observe instances in which fibrillation of the auricles set in without subjective sensations in the patient. These cases may find their explanation in those of our experiments in which the general level of pressure and the range of pulse pressure did not change in the smaller vessels. This is im-

portant because the maintenance of the pressure level at this location is one of the functions on which the sufficiency of the circulation ultimately depends. If hearts actually undergo this violent change in mechanism without necessary change in pressure, which our experiments show is possible, the fact that patients undergo the same change without sensation is explicable. That the change is accompanied by severe reaction in many individuals is, of course, well known, and in them, as Lewis and we ourselves found, temporary fall in pressure is easily assumed. The influence of ventricular rate, as Lewis has pointed out, is an important factor under experimental conditions. How important it is within the limits in which it fluctuates in patients we are not in a position to say.

#### SUMMARY.

1. Experiments on dogs show that whereas the pulse pressure in the larger arteries (femoral) varies extensively, it varies within narrow limits only in the small ones (dorsal artery of the foot).

These results supply an experimental explanation for the fact that in man uniform pressure readings were obtained by Gaertner's method at the digital artery.

2. The experiments show likewise that in certain instances the level of pressure is maintained when fibrillation of the auricles sets in.

It is therefore clear that when the mechanism of the heart beat in man changes to fibrillation of the auricles, a change, that is a fall, in pressure need not necessarily develop.

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## EXPLANATION OF PLATE 11.

FIG. 1. The effect of fibrillation of the auricles on the pulse pressure in the right femoral artery and in the dorsal artery of the left hind foot. From above down the figure records the signal of stimulation of the right auricle, the blood pressure of the right femoral artery taken by a membrane manometer the blood pressure from a dorsal artery of the left hind foot, the time in seconds.







FIG. 1.

(Cohn and Lundgaard: Peripheral blood pressure.)



## STAGGERS IN SHEEP IN PATAGONIA.\*

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PLATES 62 TO 65.

(Received for publication, August 21, 1917.)

### INTRODUCTION.

During the past few years a nervous disorder of sheep has become more prevalent throughout portions of Patagonia. This disorder has been given a number of names; among the more common may be mentioned staggers, temblique, loco, and huecù. From present reports the disease seems to be widespread, for it exists throughout the pampa at least as far north as the Chubut Valley and extends southward to Deseado and from the eastern boundary of the pampa to the Andes. The incidence varies greatly with the condition of the food supply; when there is a liberal amount of grass the actual number of cases is small. After a long continued drought when the fine grass supply is short, the number of sick animals is large. The mortality varies considerably, young sheep seeming to suffer most. The disease is not confined to the ovine species alone; horses and cattle succumb readily to it.

Acosta, in his publications on huecù,<sup>1</sup> discusses in detail the geographical distribution, symptoms, diagnosis, and prognosis of a disease similar to staggers.

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\* The field expenses of this investigation were borne by the Lochiel Sheep-Farming Company, Ltd., of London, which gave every facility for the work on its estancia at Camerones, Patagonia. Here a small bunk house close to the settlement was converted into a temporary laboratory. Only the most necessary equipment and supplies were transported from New York.

<sup>1</sup> Acosta, J. L., Producción experimental de una enfermedad de tipo nervioso, *Rev. Zool.*, 1914, v, 3; El "Huecù" o "Huaicù." Enfermedad de tipo nervioso propia de los herbívoros de la Patagonia, Tesis Universidad Nacional de Buenos Aires, No. 86, 1914.

His experimental feedings of a coarse tuft pampa grass (*Poa denudata*) to a horse, a bullock, and two sheep resulted in the production of definite nervous symptoms. Acosta's experiments were not controlled and inoculations were not attempted. He states that symptoms develop from 12 to 24 hours after the first feeding.

Conversation with a number of ranchers has given us some valuable data. The manager of an estancia in Chubut Territory supplied us with many interesting facts concerning staggers. On this particular farm about 1,000 sheep are pastured on a square league (9 square miles). The estancia consists largely of pampa<sup>2</sup> broken by many deep valleys. The water supply is excellent and consists of fresh springs coming from the hillsides close to the valley bottoms. There are many brackish water-holes as well. The herbage comprises several kinds of fine grass, two kinds of edible bush, and two coarse grasses, as well as several species of thorny bushes and a number of cacti.

Sheep were brought to this land in 1897. The land was at first unfenced and the sheep were herded. Lambs after weaning could be turned on virgin pasture. Even early in the history of the farm, employees would occasionally report a case of staggers, but it was only after the land had been fenced and animals permitted to feed over it for several years that the disease really became a menace. Beginning in 1910 more or less serious outbreaks of the disease have occurred annually. It affects lambs and hoggets (sheep under 1 year old), although adult sheep suffer to some extent. As a rule, the ewes lamb in August and the lambs are weaned in January and are then removed to separate paddocks. Early in February the malady may become epidemic and it may continue during the ensuing months until September. The incidence varies considerably with the general conditions of the pasture. When the seasons have been unusually dry and the herbage is sparse it is not unusual to see lambs affected at marking time.<sup>3</sup> The disease often exists at shearing in December. Under these conditions the nourishment has been insufficient since birth and the young have been forced to feed on grasses otherwise unnecessary.

<sup>2</sup> The term pampa in Patagonia is applied to the sandy plain beginning in the foot-hills of the Andes and sloping eastward toward the Atlantic Ocean. The vegetation of these plains is much sparser than that of those to the north.

<sup>3</sup> When lambs are 6 weeks old they are docked, marked, and castrated.

When there has been plenty of moisture and the grasses are sufficient, cases of staggers are not usually seen, either at lamb-marking or shearing, but a few cases may be observed in March and April. In extremely bad seasons the incidence may reach 100 per cent in certain paddocks. At this particular estancia the average mortality is about 5 per cent but in one paddock it reached 25 per cent.

In horses and cattle, especially in the wild herds, the losses from staggers are severe. Foals and calves from 4 to 9 months old are especially susceptible. In all paddocks located on the pampa the disease abounds. There are a few low lying paddocks, 200 or 300 feet lower than the pampa, where the disease has never existed. Sheep farmers whose lands occupy the low belt of land between the pampa and the sea declare that staggers is unknown in their flocks, although only a five strand wire fence may separate them from heavily infected pastures.

A sheep farmer whose estancia was located at Lago San Martin, about 250 leagues southwest of Camarones, informed us that his land would support 3,000 sheep to the league. Sheep had been grazed on the land for 7 years. During the first years staggers did not exist, but as the grasses became more sparse staggers became prevalent although not alarming. He stated that the young animals were affected shortly after weaning. The greatest number of cases occurred in the paddocks close to the settlement when large numbers of sheep were pastured temporarily during shearing and dipping. The fine grass in these paddocks had become very close. When questioned about the prevalence of a certain grass he informed us that it grew in all of his paddocks except those that had an elevation of over 1,500 feet. The average elevation of the paddocks was about 900 feet. Further questioning brought out the important point that he had never seen the grass commonly called pampa or coiron growing on the pampa below Deseado.

#### *Description of the Disease.*

At first sight the animal may appear normal. Excitement becomes great when the individual is alarmed by the barking of a dog, voices, etc. On becoming frightened it stands with a wild, excited look. The

neck is extended and there is usually a marked trembling of the head. Muscular twitchings of the hind legs are a constant symptom; if the animal is driven it usually breaks into a panic-stricken run. After running a short distance, stiffness of the limbs becomes marked. This stiffness is usually more noticeable in the hind legs. It moves with short, convulsive strides, and suddenly plunges forward, falling with the hind legs extended backward, and often rolls on its side (Figs. 1 to 4). If the sheep falls on a hillside it is not unusual for it to roll over and over until a level plain is reached. It is quite common to observe a sudden stiffness of all four legs; when this occurs the individual may fall directly on its side. When the animal falls it displays extreme excitability; the eyes bulge and the pupils are dilated. The head is drawn back, the muscles of the neck are tense, and the legs are extended rigidly from the body, with the digits spread far apart. If a sharp sound is made the muscles become more rigid. If the sheep is permitted to lie undisturbed the muscles gradually become flaccid, and the animal rises to its feet with some difficulty and moves away, stiffly at first, but the gait soon becomes normal. If it is frightened the same phenomena are repeated. Often there is an impairment of vision; animals may shy away from fences or attempt to jump over objects which are at a considerable distance.

When an animal has fallen and through weakness is unable to rise, it is usually found lying on its side (Fig. 5). At the slightest alarm it will make peculiar cantering movements of the legs until seized by the usual convulsions. It is not unusual to see a considerable excavation which has been made by the feet of such an animal. Often they are killed or mutilated by birds or carnivora.

Even in advanced cases of the disease, the temperature remains normal. The pulse is usually regular and weak. A ramiform congestion of the vessels of the conjunctiva is ordinarily present and the conjunctivitis is accompanied by a mucopurulent exudate. The sick animals eat well when the opportunity is afforded. There is no diarrhea or constipation.

### *Morbid Anatomy.*

*Macroscopic.*—The following autopsy notes reveal the usual type of lesions found:

*Subject.*—Yearling hogget; wether; had been down at least 2 weeks. Pulse 68; temperature 101°F.; respirations 21. Blood: hemoglobin 90 per cent; red cells 10,608,000; leukocytes 11,500. Animal killed by chloroform; much emaciated.

*Heart.*—The pericardium appears normal. The heart muscle is apparently normal. The right auricle and the right ventricle contain large dark red blood clots. The left ventricle contains a smaller clot. All the valves are normal.

*Lungs.*—Right: On the surface of the middle lobe is a dark purplish red discoloration measuring 9 by 6 cm., rather clearly demarcated from the usual bright pink lung tissue. On section it is found to involve the pleura and invade the subpleural tissue for a distance of 0.1 cm. The rest of the lung is normal. Left: Scattered over the posterior lobe of the left lung are many irregular, raised, indistinct, grayish white areas varying in diameter from 0.1 to 2 cm. They are discrete and crepitate on pressure.

*Liver.*—The liver is dark red in color and somewhat firm. On section the color is dark red. The consistency appears normal. The gall bladder is filled with dark bile.

*Spleen.*—Apparently normal.

*Kidneys.*—Right: Normal in size and reddish gray in color when viewed through the capsule, which peels off readily. On section the consistency is normal. The cortex and medulla are congested. Left: The left kidney presents the same picture as the right.

*Pancreas.*—The pancreas is slightly congested but otherwise normal.

*Esophagus and Stomachs.*—All appear normal.

*Duodenum.*—The vessels of the duodenum are congested. The mucosa is congested. The content is largely composed of mucus. The ileum, jejunum, cecum, and colon appear normal.

*Brain.*—The vessels of the dura mater are slightly congested. The superficial vessels of the cerebrum, cerebellum, and medulla are all highly congested. Along the anterior border of the cerebrum and extending 1.5 cm. on both sides of the median fissure is a triangular smoke-colored discoloration which is superficial and extends backward, reaching an apex 2 cm. from the anterior border. On the ventral aspect of the cerebrum, almost exactly opposite the dorsal

area, is another irregular patch of the same color, which measures 2 by 1.3 cm.<sup>4</sup> On section the deeper vessels are congested.

The cerebrospinal fluid is pinkish gray in color. The spinal cord is slightly congested. The sciatic nerve appears normal.

In most animals autopsied the lesions were of about the same type. It is unusual to observe congestion of the duodenum. In a few individuals small hemorrhages had occurred about the superficial vessels of the brain. The cerebrospinal fluid varied from straw color to pinkish gray. In all cases there was a more or less marked congestion of the kidneys with a certain amount of granular degeneration of the cortex, and, in some instances, of the medulla.

The intestinal tract was always examined for worms. In only two instances were they found and then only in small numbers. Examination of the frontal and nasal sinuses usually revealed varying numbers of the larvæ of *Æstrus ovis*. These were found as frequently in normal sheep as in those suffering with staggers.

Small pieces of muscle from the fore and hind legs and from the diaphragm were examined with M'Gowan's method for the presence of Sarcosporidia.<sup>5</sup> They were present in only two samples and then in very slight numbers. Frozen section of these muscles failed to reveal the parasites to any great extent.

*Microscopic.*—Pieces of various organs from normal sheep and those suffering from spontaneous and experimental staggers were fixed in Zenker's fluid. The sections were stained with methylene blue and eosin. The changes noted are slight and not specific.

The cardiac and skeletal muscles contain Sarcosporidia in all classes of sheep examined.

The liver as a rule appears normal. The kidneys are usually pathologic. The lesions vary considerably from congestion to cloudy swelling. The cortex is usually the seat of the processes.

The brain is generally congested throughout. The meninges appear normal except for a submeningeal black pigment.<sup>4</sup> The nerve cells

<sup>4</sup> At first these discolorations were regarded as possible lesions of staggers, but they were found in normal brains as frequently as in the brains of sick animals.

<sup>5</sup> M'Gowan, J. P., Investigations into the disease of sheep called "Scrapie," with especial reference to its association with sarcosporidiosis, *Edinburgh and E. Scotland Agric. Rep.*, 1914.



fail to show degenerative changes when stained with methylene blue. Congestion of the spinal cord is usually noted. In several instances small hemorrhages were visible within the gray matter. Too much stress cannot be placed on them, however, as the animals were usually killed by bleeding from the jugular veins and carotid arteries.

The spleen, lymph glands, lungs and trachea, pancreas, and adrenals fail to reveal abnormalities.

*Bacteriological Findings.*—Although inoculations from the internal organs and the central nervous system were made into various media and incubated both aerobically and anaerobically, in the main the cultures remained sterile. In animals that had been down for indefinite periods it was not uncommon to find several species of cocci in the brain, spinal cord, and cerebrospinal fluid.

Great care was taken in obtaining portions of the brain for cultivation. The skin was dissected away and the skull flamed with a gasoline blow-torch. A large sterile trephine was used for drilling the skull. Various portions of the brain were removed through the trephined holes. The cerebrospinal fluid was drawn immediately into sterile pipettes. Usually 15 cm. of the spinal cord were removed for inoculation into various media.

Films from the internal organs, brain, and spinal cord were prepared and stained with various aniline dyes. Bacteria were not uniformly present in any of them.

#### EXPERIMENTAL.

In order to ascertain whether this disease was transmissible from one animal to another, a series of experiments was undertaken. Although indications in the field seemed to point to some other etiological factor than microparasites, we undertook to establish this point definitely.

Our first series of five experiments consisted in attempts to transmit the disease by natural means and by inoculation.

*Experiments 1 to 5.*—Susceptible sheep were permitted to pasture with those suffering from staggers. The normal sheep remained so and those affected with the disease completely recovered.

Five yearling sheep and two young lambs were inoculated with normal salt

solution suspensions prepared from the central nervous system, viscera, and blood of sheep suffering from advanced staggers. The experimental animals were inoculated in various ways, some intraperitoneally, others intravenously, subdurally, and directly into the cerebral substance. The latter died promptly from shock. Chloroform anesthesia was resorted to when inoculations were made beneath the dura mater and into the brain substance. In a few of the inoculated individuals, considerable doses of the suspensions were introduced into the rumen with the aid of a stomach tube.

None of the inoculated animals developed suggestive symptoms. All remained healthy during our observation of over 2½ months. Adequate controls which were maintained throughout this series of experiments remained healthy.

Ten sheep from an outlying estancia on which staggers did not exist were purchased for experimental purposes. These animals were transported to the settlement and placed directly in disinfected pens. Some of these were used in the inoculation experiments and the others in the experimental feedings later.

Guinea pigs weighing 300 gm. were also inoculated with tissue suspensions from several cases of staggers. They remained well.

From the foregoing series of experiments it seemed well established that the disease could not be produced by permitting sick animals to come in contact with healthy ones. Moreover, in every instance when healthy susceptible sheep were inoculated with material obtained from advanced cases of staggers they failed to develop the disease.

Among certain individuals throughout the district, factors seemed to point to a grass as a possible cause for the disorder. The grass is commonly called coiron or pampa grass. Previously we have mentioned that staggers had not affected animals on low camps. The disease is confined to the pampa. It is well known that a coarse tuft grass grows in large quantities on the pampa at altitudes from 500 to 1,500 feet. The tufts may extend well down the sides of the valleys but they do not grow in the valley bottoms except close to the pampa where the canyons are shallow. It grows in tufts varying from 15 to 60 cm. in diameter. The height varies with the amount of moisture. Where the tufts have not been disturbed it may reach a height of 40 or 50 cm.<sup>6</sup>

<sup>6</sup> The writers wish to acknowledge their indebtedness to Dr. A. S. Hitchcock, Agrostologist of the Bureau of Plant Industry of the United States Department of Agriculture, who has identified this grass as *Poa argentina*.

A series of experiments was started to test the effect of this grass when fed to normal sheep and to those that had recently recovered from staggers.

*Experiment 6.*—Sept. 16, 1916. Four native yearling hoggets were placed in two small pens and fed on pampa grass. Four other animals of the same age and from the same flock were fed on alfalfa hay and served as controls. The water supply was the same in all the pens. The following notes were made during the experiment:

Sept. 20. Sheep 1 shows muscular trembling. Sept. 25. Sheep 1 is very excitable; marked weakness of hind legs; falls on becoming violently excited. Sept. 30. More violent symptoms exhibited by No. 1. Sheep 2 and 3 are highly excitable. Oct. 3. Sheep 1, 2, and 3 show characteristic symptoms of staggers; *i.e.*, short, jerky movements of the head from side to side. Sheep 1 on becoming excited loses use of the hind legs, crawls about with the fore legs, and drags the hind limbs (Fig. 6). Oct. 7. Sheep 4 shows slight shaking of the head, grinds the teeth, and has the characteristic stupid facial expression. Sheep 2 has become worse rapidly, showing the same symptoms as No. 1.

The notes of a complete physical examination of No. 1 were as follows (Fig. 6):

*Sheep 1.*—Half bred ewe; age 1 year. Muscular trembling, incoordination of the muscles of the hind legs, grinding of the jaws, constant defecation of hard feces during examination. The animal is slightly excited and restless. Temperature 104.8°F.; pulse 93, regular, full, and weak; respirations 116 (very warm day), rapid, shallow, and regular. The heart appears normal. The skin is loose and normal, the coat of wool excellent. The face is symmetrical with no evidence of inflammation of the lips, nostrils, or ears. There is a ramiform congestion of the conjunctiva accompanied with a mucopurulent exudate. The abdomen is distended. Micturition is frequent. Mental excitement is marked but the sensibility is normal. Muscular tremblings are constant and marked disturbances of the muscular sense are observed. The reflexes are normal.

The animal lies quietly during the examination. When assisted to rise and compelled to move, it trembles violently and appears weak. The hind legs are stiff and the fore legs are braced far apart.

Blood: hemoglobin 85 per cent; red cells 9,304,000; leukocytes 7,400.

Oct. 8. Animal 1 fell and was unable to rise. Oct. 10. Chloroformed.

*Autopsy.*—General condition good.

*Heart.*—The pericardium and heart appear normal.

*Lungs.*—Right, normal; left, contains one darkened area in the ventral lobe measuring 9 by 5 cm. It is clearly demarcated from the rest of the normal tissue. The lobe is somewhat congested.

*Spleen.*—Normal in size and consistency.

*Liver.*—Normal in size; the color is light brownish pink and the consistency firm. Superficially the intralobular markings are distinct. When cut the

liver appears firm and the lobules stand out clearly. The gall bladder is filled with bile.

*Kidneys*.—Both appear congested, otherwise normal. The bladder is empty. The suprarenals are normal.

*Pancreas*.—Apparently normal.

*Stomachs*.—All contain more or less partially digested grass; no evidence of inflammation.

*Intestines*.—The bowels appear normal until the jejunum is reached. Here the vessels are congested. The mucosa contains a few hemorrhagic areas varying in size from 0.2 to 0.4 cm.

*Ovaries*.—Apparently normal.

*Uterus*.—Contains a large well developed fetus.

*Brain*.—The meninges appear normal. The superficial vessels of the brain are engorged with blood. There is a grayish black discoloration on the anterior portion of both lobes of the cerebrum. This discoloration begins a distance of 2 cm. posterior to the anterior border and extends forward and downward to the ventral aspect. It is clearly demarcated from the rest of the tissue and lies only on the surface. The cerebellum and medulla appear normal. There is a normal amount of clear straw-colored cerebrospinal fluid. The superficial vessels of the spinal cord are congested.

*Bone Marrow*.—The bone marrow of the humerus and the femur appear normal. The marrow of the shaft is a dull pink color and of a stiff gelatinous consistency. That of the heads is spongy and bright red in color.

In certain tubes of the media inoculated with small pieces of brain, cocci appeared after incubation. All inoculations from the heart and liver remained sterile. Salt solution suspensions of various organs of this animal were used to inoculate the sheep employed in Experiment 5.

The other animals in the experiment gradually became worse, all developing symptoms of advanced staggers, finally falling down and becoming unable to rise.

Oct 25. Sheep 3 fell down. Nov. 7. Sheep 2 and 4 unable to rise.

The autopsies of these animals failed to show any more characteristic lesions than those found in Sheep 1. Although careful examinations of the intestines and stomachs were made, intestinal worms were not found. A common occurrence among the sheep autopsied was the presence of the larvæ of *Æstrus ovis* in the frontal sinuses.

In all these animals examinations of the muscles with M'Gowan's method for the presence of Sarcosporidia were made. In one or two instances they were present, but in very small numbers.

Unfortunately the preceding experiment was carried out with native stock. Doubtless these animals had been exposed to the disease. The results seemed to justify a repetition of the experiment with the substitution of animals which had never been exposed to staggers.

*Experiment 7.*—Oct. 5, 1916. The sheep used were of the same lot as those described under the inoculation experiments. Adequate controls were kept.

Within 3 days after starting to feed the grass one animal exhibited suspicious symptoms. 2 days later two animals revealed characteristic symptoms of staggers. The other two sheep developed staggers 10 and 15 days respectively after the feeding had been begun.

One animal (No. 5) after showing distinct symptoms of staggers was unable to maintain itself on the grass and was therefore killed. The others became progressively worse, falling down at the slightest excitement (Figs. 7 and 8).

The following results were noted when the urine of the three surviving animals was examined.

*Sheep 6.*—Amount, 10 cc.; reaction alkaline; color golden yellow, turbid; albumin present; sugar, bile, blood, and casts absent.

*Sheep 7.*—Amount 8 cc.; reaction alkaline; color golden yellow, turbid; albumin present; sugar, bile, blood, and casts absent.

*Sheep 8.*—Amount 8.5 cc.; reaction alkaline; color golden yellow, clear; albumin present; sugar, bile, blood, and casts absent.

All the animals were finally autopsied but failed to reveal more characteristic changes than those recorded.

In Experiments 6 and 7 the pulse, respiratory rate, and temperature of each animal were recorded before and during the experiments. The temperatures varied slightly and the rate of the pulse and respiration remained practically the same.

*Experiment 8.*—Nov. 7, 1916. In Experiments 6 and 7 the grass used was gathered from a paddock and stored in large bags until ready to feed. The grass tufts were cut close to the bottom and the whole tussock was used. A large proportion of the tufts consist of a dry, dead center surrounded by a more or less profuse belt of green freshly growing grass. Although we had never seen sheep eat this dry center, nevertheless it was believed by many that the dry portions were responsible for the disorder. To prove definitely whether this was responsible for the malady we determined to feed only green grass from young tussocks. In the young tufts the whole mass is green and only begins to dry as the seed ripens. Every morning 5 kilos of this fresh green grass were gathered and fed to two sheep of the same lot as those used in Experiment 7. Two animals fed on alfalfa in an adjoining pen served as controls.

After 2 days' feeding on the grass both animals showed suspicious symptoms; *i.e.*, twitching of the muscles of the hind limbs, slight shaking of the head, and twitching of the ears. At the end of 1 week's feeding they had developed severe symptoms of staggers (Fig. 9). 14 days after feeding had been begun both animals fell on the slightest excitement.\*

Examination of the urine gave the following results

*Sheep 9.*—Amount 45 cc.; reaction acid; color golden yellow, clear; specific gravity 1,060; albumin present; sugar, blood, bile, and casts absent.

*Sheep 10.*—Amount 150 cc.; reaction acid; color light golden yellow; specific gravity 1,021; albumin and sugar present; blood, bile, and casts absent.

Sheep 9 fell on the 15th day of feeding and could not rise. It was killed and autopsied on Nov. 22. Unusual changes were not noted.

The other animal, although badly affected with staggers, was able to stand, and after two feedings of green alfalfa it was turned out with two other sick animals (see Experiment 11).

*Experiment 9.*—Feeding of pampa grass to sheep that had recently recovered from staggers. Sept. 30, 1916. The feeding of pampa grass was begun with two sheep that had completely, and one that had partially recovered from staggers. These animals were part of a flock in which the disease had existed. They, with nine others, had been isolated in a small paddock about 2 weeks before our arrival and were to have furnished material for our investigation. Of these twelve animals, nine had recovered, two had become worse, and one had partially recovered. The partially recovered animal still showed extreme excitability and muscular trembling.

Oct. 5. One of the recovered sheep revealed unmistakable symptoms of staggers. The partially recovered animal had become much worse and fell on becoming excited. 2 days later the other hogget developed staggers. These animals all became worse and were either autopsied or used for other experiments.

When the three preceding experiments are reviewed it will be noted that of ten yearling sheep whose diet consisted of pampa grass all developed staggers. The controls fed on alfalfa hay remained well. Another striking feature is that both animals fed on fresh green pampa grass showed symptoms of the malady as rapidly as any of the others. The latter observation eliminates the theory of an infestation of the grass by a toxin-producing mold. The green grass we fed was free from mold.

It has been stated that old animals do not usually suffer from staggers unless they have been reared on low land and moved to the pampa. In instances of this kind, the losses may be severe. In the main, older animals that have been pastured on pampa cannot be said to suffer from the malady to any great extent. We are informed that the guanaco, a native member of the deer family, does not suffer from staggers. Indications on all upland paddocks visited showed that considerable coiron is eaten by animals. Often the green blades were kept well cropped. Knowing that more or less of the grass is eaten by older animals and that comparatively few cases of

staggers make their appearance in these flocks, we assumed that a tolerance to the toxic properties of the grass must have been developed. To test this point it was determined to feed the grass to older animals.

*Experiment 10.*—Nov. 14, 1916. In this experiment it was decided to feed adult animals on pampa grass. A 6 year old ewe with a 3 months old lamb, and two 2 year old wethers were fed upon pampa grass. The ewe could not support herself and the lamb on the diet and was discarded. The lamb showed symptoms of staggers within 8 days.

After 23 days of feeding on the grass, neither wether had developed staggers. Since it was necessary to return to New York, we requested the manager to continue feeding the grass and to telegraph us when the symptoms developed.

On Dec. 28, 45 days after the beginning of the feeding, he telegraphed us that both animals had staggers.

When young animals were fed on the grass, the average time for the first appearance of definite symptoms of the disease was 10 days. At the end of 23 days neither of the older animals revealed suspicious symptoms; in fact, 45 days of actual feeding were required to produce the disease. It seems that a considerable tolerance is developed in adult animals that have pastured on land on which the grass grows. The results of this experiment readily explain why these sheep do not suffer from the disorder to any great extent. In all probability the guanaco, through generations, has developed an immunity against the toxic substances of the grass.

*Experiment 11.*—In spontaneous outbreaks of the disorder the mortality is usually slight when compared with the incidence. In fact, in certain outbreaks all the animals recover if there is sufficient green fodder.

Sept. 16, 1916. Three yearling rams were brought into the settlement by the manager and placed in a small corral; these animals presented all the symptoms of staggers and would fall on becoming frightened (Fig. 10). They were of value for breeding purposes and the manager determined to treat them. Sept. 16 and 17. Each was given a pint of oatmeal gruel and permitted to eat rolled oats and both dry and green alfalfa. The bulk of the diet consisted of alfalfa hay. Within 1 week the improvement was marked; even when they were driven by dogs they did not fall. The symptoms rapidly disappeared and within 18 days all had recovered (Fig. 11).

In another instance, two animals, the lamb from Experiment 10 and a hogget from Experiment 8, together with a spontaneous case, were allowed to run in a small paddock where the finer grasses were plentiful. One of the animals could not be driven but had to be carried to the paddock; at the least excitement it

would fall and only rise with difficulty. Within 8 days all symptoms had disappeared and all three sheep had completely recovered.

*Experiment 12.*—Attempts were made to extract some substances from the grass that would cause the symptoms. Acosta was unable to state definitely whether the plant itself contained an alkaloid or whether a toxin was produced by the action of a mold. The former seemed more plausible as Patagonia is an exceedingly dry country. Moreover, the experiment in which we fed green grass which was not moldy seems to point to an alkaloid.

At first we attempted to extract a substance from the plant with alcohol, but on account of our small supply of that chemical we were compelled to discontinue this portion of the experiment.

We next attempted to extract some substance aqueously. This was done in the following manner: 3 kilos of grass were washed twice and then covered with water and allowed to boil for 2 hours. The liquid was then poured off and used to cover a second 3 kilos of washed grass. This was repeated a third time. The dark brown liquid thus obtained was evaporated to 1 liter. It was necessary to prepare a large quantity 3 or 4 days before the administration of the liquid was begun, to enable us to continue the supply.

Two yearling hoggets were chosen for the experiment. The fluid was administered directly into the rumen by means of a stomach tube. Each animal received 1 liter before feeding in the morning and another liter in the evening, so that each animal was receiving daily the infusion from 18 kilos of grass. The experiment was continued for a period of 33 days, but symptoms failed to develop in either of the sheep.

Guinea pigs, when injected intraperitoneally with heavy alcoholic or aqueous extracts of the plant, usually died within 15 minutes. Controls inoculated with similar preparations from non-toxic grasses died as promptly. It was impossible to attempt to purify the products and it seemed that these results may be accounted for by an excess of salts in the fluids.

A young hogget was injected intraperitoneally with a large dose of a heavy grass infusion. It developed peritonitis and died within 12 hours. Symptoms of staggers were not observed.

It seemed that animals affected with staggers or those that had recovered might react when a few drops of liquid thought to contain the toxic substance were placed in the eye. Both alcoholic and aqueous extracts were evaporated to dryness and the residue was taken up in distilled water. Normal, affected, and recovered animals received two drops of these solutions in each eye. All failed to react.

Probably only more refined and exhaustive chemical methods will be able to decide the nature of the substance which is responsible for the symptoms.



## DISCUSSION.

There seems to be very little doubt that staggers in sheep in Patagonia is caused by ingestion of a coarse tuft grass. This plant is called pampa or coiron grass. Its botanical name is *Poa argentina* (Fig. 12). All young sheep (yearlings) when fed on this grass developed characteristic symptoms of the disease. Besides our experimental evidence, there exists a number of other facts which corroborate our findings. Certain of these can best be illustrated by a map of an estancia on which staggers is prevalent (Text-fig. 1).

The larger paddocks consist of broken pampa; *i.e.*, pampa intersected by valleys. In all of them except the Windmill Paddock, the water supply is natural. The windmill wells have been driven. In all the upland paddocks staggers exists. The pampa grass grows well. On this property there are three low paddocks (Nos. 1, 2, and X) where this particular grass does not grow. These paddocks are at least 200 or 300 feet lower in altitude than the pampa. No matter how sparse the herbage may become in them staggers never develops in any of the sheep ranged there. This company owns a piece of land six leagues to the eastward and only a few feet above sea level; on this tract the disease does not exist and the coiron grass is not found. When a flock of 2 year old ewes was moved from these low camps to the mixed pampa paddocks, staggers broke out.

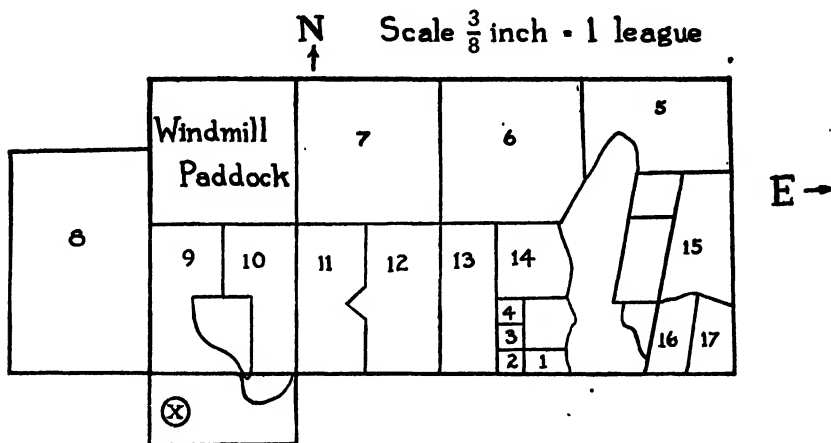
The question may be raised whether the symptoms may be caused by a lack of some vital substance in the diet; *i.e.*, whether staggers is an insufficiency disease. There are several facts which lead us to believe that the disorder is not of such a nature. When horses are brought on the pampa from other districts they often come down with staggers within 24 hours. We have seen sheep reveal symptoms after two feedings of pampa grass. In years of drought many animals die of starvation in the low camps where the pampa grass does not grow but the other food is the same. These sheep do not develop nervous manifestations.

Theiler, Green, and Viljoen<sup>7</sup> have shown that even after long periods

<sup>7</sup> Theiler, A., Green, H. H., and Viljoen, P. R., Contribution to the study of deficiency disease, with special reference to the Lamkziekte problem in South Africa, *Third and Fourth Reports, Union of South Africa, Dept. Agric.*, 1915.

horses, cattle, and sheep fed on a diet of ten parts of polished rice and one part of autoclaved hay failed to develop symptoms of an avitaminosis.

It would seem that the solution of the problem lies in the eradication of this particular grass (Fig. 13). When the fact is considered that this grass is abundant from the foot-hills of the Andes to the eastern boundary of the pampa, one gets an idea of the magnitude of the problem. During the summer months when the grass is seeding, the prevailing winds are from the west and it is not unusual for them to blow steadily for 3 or 4 days. At one time during our stay the



Paddocks 3 to 17 and the Windmill Paddock on pampa land.  
Paddocks 1 to 2 and X on low land.

TEXT-FIG. 1. Map of an estancia on which staggers is prevalent.

wind reached a maximum velocity of 84 miles an hour. Even though this grass is eradicated from eastern portions of the land it would soon reappear. One land owner tried burning off a portion of land but this failed to kill the coiron. Unless another grass can be substituted, the eradication of this particular species might work considerable havoc. There is no doubt that old sheep that have become tolerant eat it without harmful effects. Probably during periods of drought many animals are dependent on it for certain nourishment. Without the substitution of some other bulky food the losses from

starvation might well equal the losses occurring from staggers in continued periods of drought.

Individual treatment is valueless on large estancias at the present time. Many of the valleys in this district are admirably suited to the raising of alfalfa after proper treatment of the soil. Generally two good cuttings may be obtained annually after a few years. In the future when the value of sheep has advanced it may pay to cultivate portions of the better valleys and either use the alfalfa as a green forage or store it as hay or ensilage and feed it in times of stress. It is recognized at this time that this procedure is impracticable, although it may become necessary in the future.

Sheep raisers have long recognized the advisability of not exciting flocks in which many cases of staggers exist. Usually when flocks in which the incidence is heavy are driven by men and dogs, the sheep, becoming violently excited, attempt to run and many of them fall down. Of those that fall many are unable to rise and ultimately die of starvation or are killed or mutilated by animals or birds. Quiet is essential when many are sick. On the other hand, it may be advisable to move sheep to another pasture early in the outbreak if this is possible.

Acosta recommends the use of nose bags when sheep are driven through lands on which the grass exists. When it is considered that two or three men with dogs usually drive bands of a thousand or more sheep, such a procedure seems impossible.

Staggers affects horses and cattle as well as sheep. Acosta was able to produce the disease experimentally in both species by feeding *Poa denudata*. Apparently *Poa denudata* and *Poa argentina* differ in but one or two minor characteristics. Opportunity for experiments along this line was not afforded to us.

#### CONCLUSIONS.

After observations and experimental work both in the field and laboratory, the following conclusions seem justified:

1. Staggers is a non-infectious disorder affecting horses, cattle, and sheep.
2. The disease is characterized by weakness, muscular twitching, irregular movements of the head, stiffness of the limbs, and transient

motor paralysis, accompanied with spastic spasms on excitement. There is also a derangement of vision and conjunctivitis.

3. The postmortem lesions are not characteristic.

4. We readily produced the disease by feeding susceptible sheep on a coarse tuft grass commonly known as coiron or pampa grass (*Poa argentina*).

5. The time required to produce definite symptoms by feeding the grass varied. Two animals developed typical staggers after two feedings; in another instance a period of 21 days of feeding was required. The average time for the production of unmistakable symptoms in our experiments was 10 days.

6. Many sheep recover from staggers spontaneously. A complete change of diet will usually effect a cure within 2 weeks.

7. Older animals that have pastured for long periods on lands where the grass grows become tolerant and are rarely affected with staggers.

8. The grass is toxic to sheep at all seasons of the year. We fed late winter and early spring grass and grass in flower, and produced staggers in every instance. The young green grass is as toxic as any edible portion of the plant.

#### EXPLANATION OF PLATES.

##### PLATE 62.

FIGS. 1 to 5. Spontaneous staggers.

FIG. 1. Animal about to fall on its side; the fore legs are rigidly extended and the digits spread far apart.

FIG. 2. The transient motor paralysis of the hind limbs occurring after violent exercise and excitement.

FIG. 3. Sheep about to fall. All four legs are extended rigidly. The momentum gained was sufficient to carry the body forward. The animal appears to plunge forward while falling.

FIG. 4. The gait of sheep with staggers; note the apparent rigidity of the hind limbs.

FIG. 5. The usual position of an animal down with staggers.

##### PLATE 63.

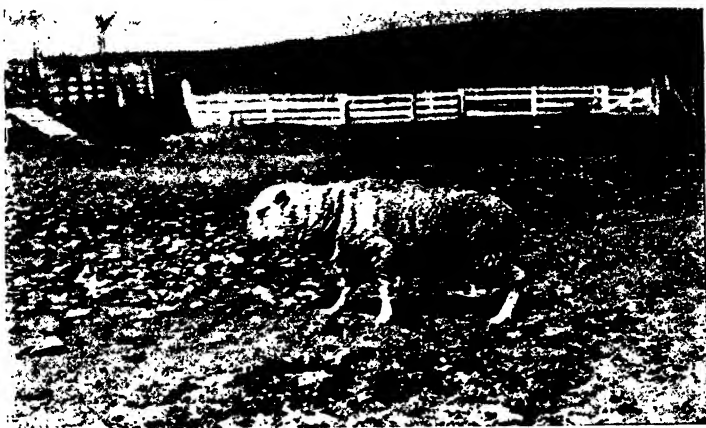
FIGS. 6, 7, and 8. Experimental staggers.

FIG. 6. Sheep 1, Experiment 6. Note the stiff carriage of the hind limbs and the depression of the hind quarters.



(Jones and Arnold: Staggers in sheep.)

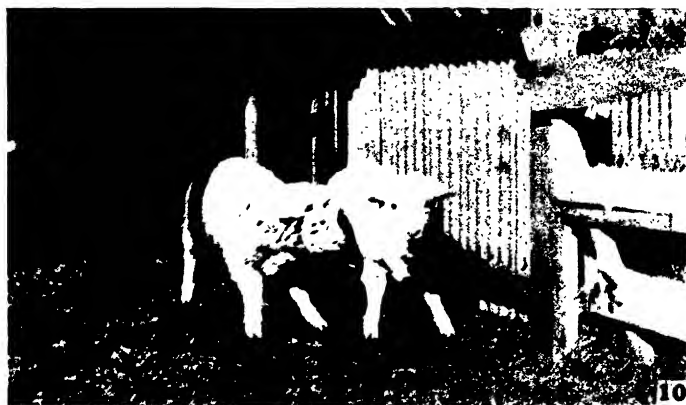




(Jones and Arnold: Staggers in sheep.)

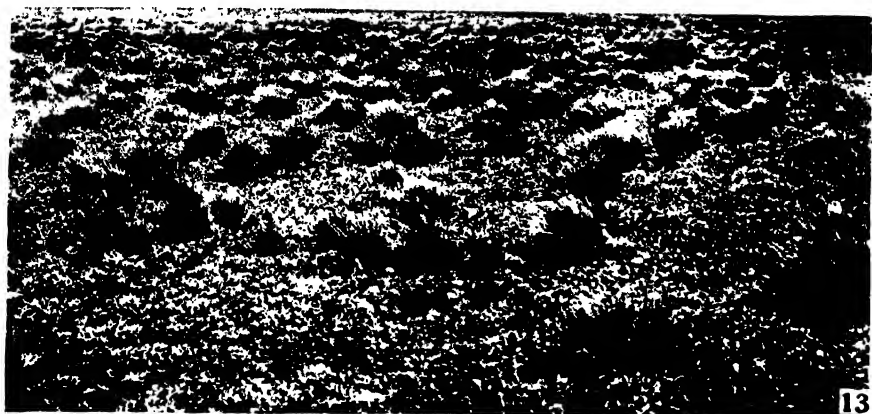
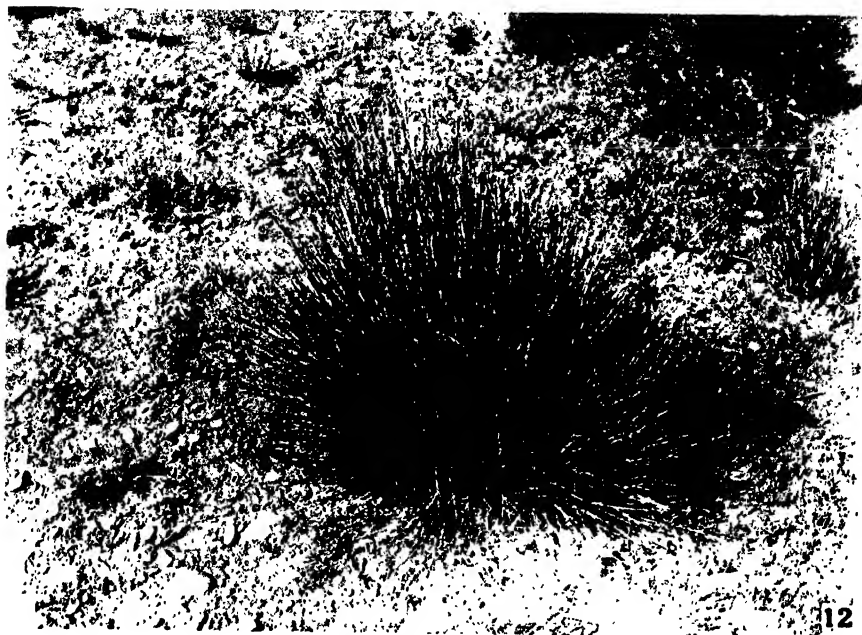






(Jones and Arnold; Staggers in sheep.)





(Jones and Arnold: Staggers in sheep.)



